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Ambient Water Quality Criteria for Hexachlorocyclopentadiene

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16. ABSTRACT

Section 304(A) of the Clean Water Act [33 U.S.C. 1314(A)], requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for Hexachlorocyclopentadiene. It presents concentration criteria for the protection of freshwater and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A Section 304(A) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility nor is it a water quality standard and in itself has no regulatory effect.

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AMBIENT WATER QUALITY CRITERIA FOR

HEXACHLOROCYCLOPENTADIENE

Prepared By
U.S. ENVIRONMENTAL PROTECTION AGENCY

Office of Water Regulations and Standards
Criteria and Standards Division
Washington, D.C.

Office of Research and Development
Environmental Criteria and Assessment Office
Cincinnati, Ohio

Carcinogen Assessment Group
Washington, D.C.

Environmental Research Laboratories
Corvalis, Oregon
Duluth, Minnesota
Gulf Breeze, Florida
Narragansett, Rhode Island

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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisifaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

STEVEN SCHATZOW
Deputy Assistant Administrator
Office of Water Regulations and Standards

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Aquatic Life Toxicology:

William A. Brungs, ERL-Narragansett
U.S. Environmental Protection Agency

David J. Hansen, ERL-Gulf Breeze
U.S. Environmentla Protection Agency

Mammalian Toxicology and Human Health Effects:

Mary Ann Bell Zanetos (author)
Battelle Columbus Laboratory

Terence M. Grady (doc. mgr.) ECAO-Cin
U.S. Environmental Protection Agency

Jerry F. Stara, (doc. mgr.) ECAO-Cin
U.S. Environmental Protection Agency

Patrick Durkin
Syracuse Research Corporation

Renate Kimbrough
Center for Disease Control

Robert McGaughy*
Carcinogen Assessment Group
U.S. Environmental Protection Agency

Myron Mehlman
Mobil Oil Corporation

Joseph Borzelleca
Medical College of Virginia

Robert Bruce, ECAO-RTP
U.S. Environmental Protection Agency

Fred Coulston
Albany Medical College

Alfred Garvin
University of Cincinnati

James Lucas, HERL
U.S. Environmental Protection Agency

H.M. Mehendale
University of Mississippi Medical Center

Herb Pahren, HERL
U.S. Environmental Protection Agency

Technical Support Services Staff: D.J. Reisman, M.A. Garlough, B.L. Zwayer, P.A. Daunt, K.S. Edwards, T.A. Scandura, A.T. Pressley, C.A. Cooper, M.M. Denessen.

Clerical Staff: C.A. Haynes, S.J. Faehr, L.A. Wade, D. Jones, B.J. Bordicks, B.J. Quesnell, P. Gray, R. Rubinstein.

*CAG Participating Members: Elizabeth L. Anderson, Larry Anderson, Dolph Arnicar, Steven Bayard, David L. Bayliss, Chao W. Chen, John R. Fowle III, Bernard Haberman, Charalingayya Hiremath, Chang S. Lao, Robert McGaughy, Jeffrey Rosenblatt, Dharm V. Singh, and Todd W. Thorslund.

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CRITERIA DOCUMENT

HEXACHLOROCYCLOPENTADIENE

CRITERIA

Aquatic Life

The available data for hexachlorocyclopentadiene indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 7.0 and 5.2 µg/l, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for hexachlorocyclopentadiene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 7.0 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of hexachlorocyclopentadiene to sensitive saltwater aquatic life.

Human Health

For comparison purposes, two approaches were used to derive criterion levels for hexachlorocyclopentadiene. Based on available toxicity data, for the protection of public health, the derived level is 206 µg/l. Using available organoleptic data, for controlling undersirable taste and odor quality of ambient water, the estimated level is 1 µg/l. It should be recognized that organoleptic data as a basis for establishing a water quality criteria have limitations and have no demonstrated relationship to potential adverse human health effects.

INTRODUCTION

Hexachlorocyclopentadiene (hex; C-56; 1,2,3,4,5,5-hexachlorocyclopentadiene) is a pale-to-greenish-yellow liquid with the molecular formula, C_5Cl_6. Other physical properties include a molecular weight of 272.77; a solubility in water of 0.805 mg/l; a vapor pressure of 1 mm Hg at 78-79°C and a density of 1.7119 (20°/4°C) (Lu, et al. 1975; Ungnade and McBee, 1958).

Hex was the key intermediate in the manufacture of the organochlorine pesticides endosulfan and Pentac® and formerly in the manufacture of several commercially important organochlorine pesticides whose usage is now banned or restricted (Kirk and Othmer, 1964). Although it has also been suggested for use as an intermediate in the manufacture of dyes, pharmaceuticals, resins, and germicides, these latter uses account for only a very small percentage of hex production. Historically, hex has been produced in the United States by two companies, Hooker Chemical and Plastics Corporation (Montague, Michigan) and Velsicol Chemical Corporation (Memphis, Tennessee). In 1977, Hooker discontinued hex manufacture at the Montague plant, making Velsicol's Memphis plant the only current U.S. producer. Hex is produced at several facilities outside the U.S. Hex was formerly used in the manfacture of aldrin, endrin, and dieldrin at the Shell Chemical Company plant in Denver, Colorado (Zavon, 1978).

Hex has been used as a chemical intermediate in the production of numerous chlorinated pesticides, several of which have enjoyed very large usage. The list includes chlordane, aldrin, dieldrin, heptachlor, isodrin, endrin, mirex, Kepone, endosulfan (Thiodan®), and Pentac®. With the exception of endosulfan and Pentac®, both of which are in current use, the usage of hex-based pesticides has been banned, suspended, or severely restricted by

governmental action. Although current production estimates are uncertain and highly variable, one estimate has placed annual production as high 50 million pounds (25,000 tons) per year (Bell, et al. 1978). Recent bans or restrictions on many of the chlorinated pesticides have led to a decline in the use of hex as a chemical intermediate in the manufacture of these products; simultaneously, the use of hex in the manufacture of flame retardants has increased. Currently, a major use of hex is in the manufacture of flame retardant compounds such as chlorendic acid and chlorendic anhydride which are produced by reacting equimolar quantities of hex and maleic anhydride. These and other hex-derived chlorinated organic compounds confer flame retardant properties to plastics, including polypropylene, polyethylene, nylon, rigid polyurethane foams, unsaturated polyesters, and other polymers including eposy resins (Sanders, 1978).

Although hex is a commercially important chemical intermediate with high annual production, it has essentially no end uses of its own. Consequently, hex concentrations in the environment should be negligible and limited data suggest that this indeed is the case. Small amounts of hex are occasionally present as impurities in pesticides made from it and some has undoubtedly entered the environment in this way. The most likely route of entry into the environment arises from the manufacture of hex or hex-containing products. Discharge of these industrial wastes appears to constitute the only documented sources of measurable hex in environmental samples.

Due to its infrequency in the environment and its low profile as a chemical intermediate, there have been few studies of the behavior of hex in the environment or in biological systems. By the same token, until recently, hex was not recognized as a major environmental problem nor a potential threat to humans (except for those occupationally exposed). A recent inci-

dent in which eighty-six workers at a sewage treatment plant in Louisville, Kentucky, experienced a variety of toxic symptoms following the improper disposal of hex manufacturing wastes has created a great demand for information concerning the effects of hex exposure on humans.

Several literature reviews on the health and environmental effects of hex are available. These include reviews of Equitable Environmental Health, Inc. (1976); U.S. Environmental Protection Agency (1977); National Academy of Sciences (1977); and Bell, et al. (1978). Although each of these reports is different in emphasis, they each note the unfortunate absence of epidemiologic studies of hex-exposed workers and the lack of suitable chronic exposure studies of animals (especially with respect to carcinogenicity). Until these types of information are available, proposed environmental criteria will necessarily be based on extrapolation of animal data to humans, a practice which is invariably speculative and prone to error. Perhaps more importantly, in the absence of suitable chronic exposure studies, recommendations must be based on avoidance of relatively overt manifestations of toxicity (e.g., abnormalities in physiologic tests, increased incidence of neoplasms, etc.) which may manifest themselves only after years of exposure. Since effects of the latter type tend to be elicited at doses lower than those causing acute toxicity, criteria based on acute responses may fail to provide adequate protection. Consequently, the criterion levels suggested in this document are presented with the understanding that they are based on decidedly inadequate chronic effects data and should be reassessed upon completion of appropriate chronic studies.

Several transport and fate processes appear to operate at significant rates to remove hexachlorocyclopentadiene from aquatic systems. The relative importance of these processes is thought to depend strongly on the

characteristics of the individual water body, so that there is not clear indication that one process is predominant on an overall basis. The most important fate processes appear to be hydrolysis and near-surface photolysis, while transport occurs via the water column (as dissolved species), by volatilization to the atmosphere, and through absorption onto particulates (perhaps to a lesser extent). The fate of hex in the troposphere is not known (U.S. EPA, 1979).

Six active chlorines and two double bonds make hex a highly reactive compound which readily undergoes substitution and additional reactions. Its versatility is based upon its reactivity as a diene with a variety of olefins and polynuclear aromatic hydrocarbons in the Diels-Alder reaction.

Rieck's report (1977a) provides evidence of the volatilization of hex from soil. Vapors of ^{14}C-hex were evolved from treated soil to the extent of 11, 13, 15, 16, 17, and 19 percent (cumulative) of the applied amounts, 1, 2, 3, 5, 7, and 14 days respectively after treatment. One could, therefore, deduce that there is volatility from treated soil and that the rate decreases with time.

Another distinguishing feature of hex is that it appears to be strongly adsorbed to soil or soil components. Two studies of hex-treated soil (Rieck, 1977a,b) have demonstrated poor extractability from soil, which provides indirect evidence of strong adsorption. In one study (Rieck, 1977b), soil which had been extracted was then combusted to $^{14}CO_2$. Any residual but unextracted ^{14}C was then measured directly. Unextracted ^{14}C was found in these samples and thus was accounted for as a "bound" residue. Had it not been accounted for, it would have probably been assumed to have volatilized.

Hex, unlike some of the pesticides derived from it, degrades rapidly by photolysis, giving water soluble degradation products. Tests on its stability towards hydrolysis at ambient temperature indicated a half-life of about 11 days at pH 3-6, which was reduced to 6 days at pH 9. In another experiment (Bevenue and Yeo, 1969), the vaporization and absorption properties of hex in organic solvent (iso-octane) and in aqueous media were examined preparatory to studying the adsorptive effects of the chemicals on stored foods. Gas chromatographic data from the solutions of distilled water containing the adsorbed vapor of hexachlorocyclopentadiene revealed that the chemical had completely disappeared after three days exposure, indicating dissipation or decomposition.

Data from the iso-octane solutions revealed no degradation after 24 hours, but a multi-peak spectrum indicating the presence of degradation products was obtained after 7 to 21 days' exposure. This spectrum suggested to the investigators that the compound may be susceptible to atmospheric oxidation and/or photodecomposition (National Cancer Institute, 1977).

In using hex as an intermediate in the manufacture of various chlorinated pesticides (chlordane, dieldrin, heptachlor, etc.), it appears that although yields in all reactions are good, they are not quantitative. Thus, there is reason to suspect that in some cases free hex may have been present in the marketed pesticide products. An early study by Ingle (1953) provided evidence that the reported vapor toxicity of chlordane to mice was not attributable to chlordane, but to some unreacted intermediates, chief of which was hexachlorocyclopentadiene. It is suspected that small quantities of unreacted hex may be present in other related pesticides as well.

Because of the widespread use of hex as an intermediate, and the belief that hex may have at one time comprised as much as one percent of commercial

chlordane (Ingle, 1953), laboratory studies have been undertaken to determine its fate under various environmental conditions (Metcalf, et al. 1971; Lu, et al. 1975). Limited studies indicate that the chemical would not be expected to persist in the environment. For example, the bioaccumulation and degradation of ^{14}C-labeled hex was investigated in a laboratory model ecosystem, simulating the application of chemicals to plants and subsequent contamination of the aquatic environment. A 5.0 mg quantity of the labeled hexachlorocyclepentadiene was topically applied to plants in the terrestrial portion of the model and the products were allowed to pass through the entire system over a 33-day period of 80°F and with a 12-hour light cycle. The concentration of hex reached a maximum level of only 0.031 mg/l in the water phase of the model after 14 days, decreasing to 0.016 mg/l by 33 days. There was evidence of bioaccumulation of hex as indicated by its recovery as 33 percent of the extractable ^{14}C in algae, 50 percent in snail, 46 percent in mosquito, and 41 percent in fish; but the concentration of total ^{14}C in these various organisms was relatively low (compared to the other chemicals tested), indicating substantial volatility. None of the trace degradation products was identified although the extent of total degradation was estimated to be: water, 77 percent; algae, 4 percent; snail, 10 percent; mosquito, 2 percent; and fish, 37 percent. The interpretation of this data, especially with respect to biomagnification has been the subject of controversy (Whitacre, 1978).

Hex enters the environment primarily through discharges and emissions from pesticide production facilities; smaller quantities enter the environment through the use of pesticides and compounds in which hex is present as an impurity, e.g., chlordane (Harris, 1972). Once in the environment it may be transported by wind, surface and underground water, streams, and biota.

In December, 1975, hex was qualitatively identified as a contaminant in the discharge of a pesticide production plant in Memphis. Later, (May, 1977), the compound was identified in the air at the Hooker plant in Montague, Michigan (56 ppb), in its aqueous discharge (0.170 mg/l), and in fish tissue from the receiving stream (14-18 ppb) (Spehar, et al. 1977). Hex has also been reported to have been present in soil and bay sediments in the vicintiy of a Virginia pesticide plant long after production was discontinued (Swanson, 1976).

Data on environmental concentrations of hex are minimal except for industrial discharges. Velsicol Chemical Corporations's Memphis plant has been issued a National Pollutant Discharge System (NPDS) permit. Monitoring activities in connection with the discharge permit indicate that hexachlorocyclopentadiene, hexachloronorbornene, and hexachlorobornadiene are being discharged into the City of Memphis wastewater collection system (Bennette, 1977; Marks, 1977). A sampling from the month of January, 1977 (31 consecutive days), revealed hex concentrations in wastewater ranging from 0.156 to 8.240 mg/l. The U.S. Environmental Protection Agency's Water Surveillance Branch sampled Velsicol's discharge February 2-3, 1977. Hex was detected at 18 mg/l. Based on the average monthly discharge by the Velsicol Chemical Corporation during February, 1977 (3.16 million gallons per day), 474 pounds of hex were believed to have been discharged through Velsicol's discharge outfall into the City of Memphis Wastewater Collection System and then into the Mississippi River during the period February 2-3, 1977. Calculated on the basis of the flow rate above, this discharge caused a concentration of hex in the Mississippi River of 0.0006 mg/l (Carter, 1977).

In a recent, well-publicized incident, an estimated 6 tons equivalent of hexachlorocyclopentadiene (hex) and octachlorocyclopentene (octa) dispersed

in No. 4 fuel oil were dumped into the Louisville, Kentucky, municipal sewer system's Western Outfall sewer. The contaminated sludge entered the Morris Forman Wastewater Treatment Plant on March 26, 1977, causing illness among sewage treatment plant workers. Toxic effects associated with this episode forced closure of the plant with subsequent diversion of 105 million gallons per day of raw sewage into the Ohio River. There was no evidence of environmental release (outside the immediate environs of the sewage treatment plant and contaminated sewer lines). It was, however, necessary to decontaminate the sewer system and the treatment plant.

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Aquatic Life Toxicology*

INTRODUCTION

Freshwater acute data are available for several fish species and hexachlorocyclopentadiene. An embryo-larval test has been conducted with the fathead minnow. Results of tests with Daphnia magna indicate that it may be more sensitive than the fish.

Acute tests with six saltwater species have been conducted and, with the exception of a polychaete, these species were similarly sensitive to this compound.

EFFECTS

Acute Toxicity

Two results are available for Daphnia magna with good reproducibility between investigators; the 50 percent effect levels were 39 and 52 µg/l (Table 1).

Henderson (1956) exposed the fathead minnow under three different conditions using two dilution waters. One test water had a hardness of 40 mg/l and pH of 7.4, and the second test water had a hardness of 400 mg/l and pH of 8.2. Two tests with hard water were conducted to evaluate the method used to add the chemical to the dilution water. The latter comparison was important since hexachlorocyclopentadiene is quite volatile and has an extremely low solubility in water. The chemical was added in a 0.01 percent acetone solution or a 0.001 percent suspension of an emulsion prepared in a blender. The effect of hardness, if any, was slight with 96-hour LC_{50} values of 104 µg/l in soft water and 78 µg/l in hard water (Table 1). The

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*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

test results comparing methods of addition were 78 and 59 µg/l (Table 1) indicating little difference. Spehar, et al. (1979) also determined a 96-hour LC_{50} value for larval fathead minnows; this result, using flow-through procedures and measured concentrations, was 7.0 µg/l (Table 1). The difference between the data of Henderson (1956) and Spehar, et al. (1979) may be due to differences in test methods or in relative sensitivity of different life stages of the fathead minnow; the species acute value is 7.0 µg/l since there is only one flow-through test with measured concentrations. The channel catfish and bluegill 96-hour LC_{50} values indicate that they are similar to the fathead minnow in sensitivity to hexachlorocyclopentadiene (Table 1).

Of the six saltwater species for which LC_{50} values are available, the polychaete was most resistant with a 96-hour LC_{50} of 371 µg/l (Table 1). The other two invertebrate species were similar in sensitivity to the three fish species with a range of LC_{50} values from 32 to 48 µg/l for the five species tested under static conditions. The mysid shrimp was also tested under flow-through conditions and measured concentrations, giving a 96-hour LC_{50} of 7.0 µg/l. The related static result was 32 µg/l. This comparison indicates that static procedures will probably underestimate the toxicity of hexachlorocyclopentadiene.

Chronic Toxicity

The chronic value for the fathead minnow embryo-larval test by Spehar, et al. (1979) is 5.2 µg/l (Table 2). This concentration is not much lower than the 96-hour LC_{50} value (7.0 µg/l) for larval fathead minnows and results in an acute-chronic ratio of 1.3 (Table 2).

Plant Effects

No data are available on the effects of hexachlorocyclopentadiene on freshwater or saltwater algae or plants.

Residues

The bioconcentration factor for whole-body fathead minnows is 11 (Table 3) after a 30-day exposure (Spehar, et al. 1979). No Residue Limited Toxicant Concentration can be determined since there is no permissible tissue residue concentration available.

Miscellaneous

Applegate et al. (1957) exposed sea lamprey, rainbow trout, and bluegill to concentrations of hexachlorocyclopentadiene of 1,000 and 5,000 µg/l (Table 4). Death or distress was observed in one-half to one hour. The 30-day LC_{50} value for the fathead minnow (Spehar, et al. 1979) is 6.7 µg/l which result is only slightly lower than the 96-hour LC_{50} value of 7.0 µg/l determined by the same investigators.

Summary

Hexachlorocyclopentadiene is very toxic to freshwater organisms. Under static conditions, 50 percent effect concentrations for Daphnia magna and three fish species were in the range of 39 to 180 µg/l. A comparison of static and flow-through conditions indicates that the latter yields significantly lower lethal values. The chronic value for the fathead minnow was 5.2 µg/l, a concentration only slightly below a lethal concentration for that species. Residues of hexachlorocyclopentadiene do not appear to be a problem with a bioconcentration factor in fish of 11. The 30-day LC_{50} value for the fathead minnow was 6.7 µg/l, a concentration only slightly lower than the flow-through 96-hour LC_{50} of 7.0 µg/l.

The saltwater data base is more limited, with 96-hour LC_{50} values for three invertebrate and three fish species obtained under static conditions in the range of 32 to 48 µg/l for all species except the polychaete for

which the LC_{50} value was 371 µg/l. As with the fathead minnow, the flow-through LC_{50} for the mysid shrimp was significantly lower than the static test result with the same species. No other data are available for saltwater organisms.

CRITERIA

The available data for hexachlorocyclopentadiene indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 7.0 and 5.2 µg/l, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for hexachlorocyclopentadiene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 7.0 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of hexachlorocyclopentadiene sensitive saltwater aquatic life.

Table 1. Acute values for hexachlorocyclopentadiene

Species	Method[a]	LC50/EC50 (µg/l)	Species Mean Acute Value (µg/l)	Reference
		FRESHWATER SPECIES		
Cladoceran, *Daphnia magna*	S, U	39	--	EG & G, Bionomics, 1977
Cladoceran, *Daphnia magna*	S, U	52	45	Union Carbide Environmental Services, 1977
Fathead minnow (larva), *Pimephales promelas*	FT, M	7.0	--	Spehar, et al. 1979
Fathead minnow, *Pimephales promelas*	S, U	104	--	Henderson, 1956
Fathead minnow, *Pimephales promelas*	S, U	78	--	Henderson, 1956
Fathead minnow, *Pimephales promelas*	S, U	59	--	Henderson, 1956
Fathead minnow, *Pimephales promelas*	S, U	180	7.0	EG & G, Bionomics, 1977
Channel catfish, *Ictalurus punctatus*	S, U	97	97	EG & G, Bionomics, 1977
Bluegill, *Lepomis macrochirus*	S, U	130	130	EG & G, Bionomics, 1977
		SALTWATER SPECIES		
Polychaete, *Neanthes arenaceodentata*	S, U	371	371	U.S. EPA, 1980
Mysid shrimp, *Mysidopsis bahia*	S, U	32	--	U.S. EPA, 1980
Mysid shrimp, *Mysidopsis bahia*	FT, M	7.0	7.0	U.S. EPA, 1980
Grass shrimp, *Palaemonetes pugio*	S, U	42	42	U.S. EPA, 1980

Table 1. (Continued)

Species	Method*	LC50/EC50 (µg/l)	Species Mean Acute Value (µg/l)	Reference
Sheepshead minnow, *Cyprinodon variegatus*	S, U	45	45	U.S. EPA, 1980
Pinfish, *Lagodon rhomboides*	S, U	48	48	U.S. EPA, 1980
Spot, *Leiostomus xanthurus*	S, U	37	37	U.S. EPA, 1980

* S = static, FT = flow-through, U = unmeasured, M = measured

Table 2. Chronic values for hexachlorocyclopentadiene (Spehar, et al. 1979)

Species	Method*	Limits (µg/l)	Chronic Value (µg/l)
FRESHWATER SPECIES			
Fathead minnow, Pimephales promelas	ELS	3.7-7.3	5.2

* ELS = early life stage

Acute-Chronic Ratio

Species	Acute Value (µg/l)	Chronic Value (µg/l)	Ratio
Fathead minnow, Pimephales promelas	7.0**	5.2**	1.3

** These two values were selected to calculate the acute-chronic ratio because both tests were conducted in the same dilution water (Lake Superior) and this acute value is the only one for the fathead minnow that was obtained under flow-through, measured test procedures.

Table 3. Residues for hexachlorocyclopentadiene (Spehar, et al. 1979)

Species	Tissue	Bioconcentration Factor	Duration (days)
	FRESHWATER SPECIES		
Fathead minnow (juvenile), *Pimephales promelas*	whole body	11	30

Table 4. Other data for hexachlorocyclopentadiene

Species	Duration	Effect	Result (µg/l)	Reference
		FRESHWATER SPECIES		
Sea lamprey (larva), *Petromyzon marinus*	24 hrs	Death in 1 hr	5,000	Applegate, et al. 1957
Sea lamprey (larva), *Petromyzon marinus*	24 hrs	Distress in 1/2 hr	1,000	Applegate, et al. 1957
Rainbow trout (fingerling), *Salmo gairdneri*	24 hrs	Death in 1/2 hr	5,000	Applegate, et al. 1957
Rainbow trout (fingerling), *Salmo gairdneri*	24 hrs	Death in 1 hr	1,000	Applegate, et al. 1957
Fathead minnow (larva), *Pimephales promelas*	30 days	LC50	6.7	Spehar, et al. 1979
Bluegill (fingerling), *Lepomis macrochirus*	24 hrs	Death in 1/2 hr	5,000	Applegate, et al. 1957
Bluegill (fingerling), *Lepomis macrochirus*	24 hrs	Distress in 1/2 hr	1,000	Applegate, et al. 1957

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Mammalian Toxicology and Human Health Effects

EXPOSURE

Ingestion from Water

Very little is known regarding potential hex exposures through ingestion of contaminated food or water. Hexachlorocyclopentadiene (Hex) has been detected in specific bodies of water near points of industrial discharges. Except for such source-directed sampling, it appears that there is little information on hex concentrations in surface waters. Hex is usually not detectable in water samples. Due to its low solubility and tendency to volatilize, one would not expect it to remain in flowing water. Moreover, there are no data on hex levels in drinking water or the extent to which hex in raw (untreated) water would be passed through the water treatment process to human consumers.

Ingestion from Food

Hex has been identified in a few samples of fish taken from waters near the Hooker plant in Michigan (Spehar, et al. 1977). Frequently, however, hex residues have not been detected in edible fish deliberately exposed to hex in laboratory experiments. According to the same investigator, the inability to recover hex in fish samples probably results from losses by vaporization during sample extraction. No reports concerning hex contamination of other foods could be located (Spehar, et al. 1977).

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid soluble com-

pound in the tissues of various aquatic animals seem to be proportional to the percent lipid in the tissue. Thus, the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

A measured steady-state bioconcentration factor of 11 was obtained for hexachlorocyclopentadiene using fathead minnows (Spehar, et al. 1979). Similar fathead minnows contained an average of 7.6 percent lipids (Veith, 1980). An adjustment factor of 3.0/7.6 = 0.395 can be used to adjust the measured BCF from the 7.6 percent lipids of the fathead minnow to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for hexachlorocyclopentadiene and the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 11 x 0.395 = 4.34.

Inhalation

The heaviest and most chronic exposure to hex undoubtedly occurs among persons engaged directly in the manufacture of hex and among production workers fabricating hex-containing products. Although several cohorts of hex-exposed workers can be specifically identified (employees of Hooker Chemicals and Plastics, Michigan and Niagara Falls plants; Velsicol Chemical Corporation, Memphis plant; Shell Chemical Company, Denver and Pernis, Netherlands, plants; an Israeli company, Makhteshim; and the Hooker plant at Genk, Belgium), there have been no reports of epidemiologic studies of these workers. Hooker Chemicals and Plastics Corporation, a manufacturer of hex, reported that they are presently conducting a mortality study of chronically exposed hex workers, but the study is in its initial stages and very likely will not be completed until 1980 (Zavon, 1978, personal communication). Inhalation of hex is the primary mode of occupational exposure. Accidental spills and illegal discharges of hex represent the primary mode of acute human exposure (e.g., the Louisville incident). Inhalation appears to be the most important mode of exposure in these cases as well.

Dermal

According to Hooker Chemical and Plastic Corporation's Material Safety Data Sheet, hex is readily absorbed through the skin. Prolonged or repeated contact can lead to burns and manifestations of systemic toxicity not unlike those caused by inhalation. The hazards of skin contact are well recognized and industrial workers are provided with impervious clothing to prevent dermal contact

(Hooker, 1972). Thus, dermal exposure should not be anticipated among workers familiar with hex. Persons outside the chemical industry can be exposed to hazardous contacts as a result of accidental spills or improper disposal of hex.

PHARMACOKINETICS

Two studies which address the pharmacokinetics of hex could be located (Mehendale, 1977; Dorough, 1979). The Mehendale study focuses upon the disposition of hex within the body and its modes of elimination, and the Dorough study reported accumulation, distribution, and elimination of hex in mice and rats.

In the Mehendale (1977) study, radiolabeled hexachlorocyclopentadiene (^{14}C-hex) was administered by oral intubation to four male Sprague-Dawley rats in order to examine absorption, metabolism, and excretion of the compound following a single oral dose. After dosing with ^{14}C-hex (5 µmoles, 1 µCi per animal), the rats were maintained in metabolism cages for seven days, during which daily urine and fecal samples were collected. After seven days, the animals were sacrificed and the major organs were removed and radioassayed.

Urine and powdered fecal samples were radioassayed for total ^{14}C. An average of approximately 33 percent of the total dose was excreted in the urine after seven days. About 87 percent of that (approximately 28.7 percent of total dose) was eliminated during the first 24 hours after the administration of the compound. Fecal excretion accounted for 10 percent of the total dose; nearly 60 percent of the 7-day fecal excretion occurred during the first day.

Beyond the third day after treatment, only trace amounts of the hex-derived ^{14}C were eliminated in the feces. Tissues retained only trace amounts of hex after seven days. For example, the kidney retained only about 0.5 percent of the total dose and the liver less than 0.5 percent. Other organs and tissues -- fat, lung, muscle, blood, etc. -- contained even less of the radiolabel. Such findings suggest that at least half of the administered hex was eliminated by routes other than urine and feces. The author felt that the respiratory tract is probably the major route of excretion.

The nature of the radioactivity excreted in the urine was examined searching for possible metabolites. It was found that about 70 percent of the radioactivity in the urine was extractable using a hexane:isopropanol (9:1) mixture. The organic solvent was concentrated, applied to thin-layer chromatography (TLC) plates, and developed in three solvent systems. The radioactive spots were visualized by auto-radiography on medical x-ray film. The results suggested the presence of at least four metabolites; however, at the time of this writing they had not been identified and characterized.

Disposition and biliary excretion of ^{14}C-hex was studied by injection of approximately 1 µCi (5 µmole) of ^{14}C-hex into the femoral vein of anesthetized rats. Timed samples of blood and bile were collected for one hour from the femoral artery and common bile duct which had been cannulated prior to dosing. Approximately 9 percent of the administered dose was excreted in the bile in one hour. Because this quantity is equivalent to that excreted in the

feces over seven days, enterohepatic circulation of this compound is probable. The nature of the compound present in the bile is not yet known.

At the end of the above experiments, the animals were sacrificed and the liver and kidneys were removed. Tissue homogenates from these organs were radioassayed and the distribution of the radioactivity among the various subcellular fractions was examined by assaying the various centrifugation fractions. Kidney cytosol accounted for 93 percent of the radioactivity in the total kidney homogenate. This behavior is consistent with rapid urinary excretion. Similarly, 68 percent of the radioactivity in the liver homogenate was associated with the liver cytosol fraction, once again consistent with rapid excretion.

Pre-exposure of some of the rats to hex (50 mg/kg/day) for three days prior to the experiment did not affect blood decay curves and biliary excretion; however, an increased concentration in the kidneys after a single challenge with ^{14}C-hex was observed.

Dorough (1979) reported a pharmacokinetic investigation of hex completed under contract to Velsicol Chemical Corporation.

In a single oral dose study, ^{14}C-labeled-hex was given by gavage to Sprague-Dawley rats and albino mice of both sexes in two dosages, 2.5 mg/kg and 25 mg/kg. Animals were kept in metabolism cages and feces and urine were collected separately. Animals were killed on days one, three and seven post-treatment. There was no appreciable difference in excretion patterns between species or sex, and the major route of excretion was through the feces with 83.4 percent of the 2.5 mg/kg dose and 85.5 percent of the 25 mg/kg

dose excreted by the third day after treatment. Rats showed maximum ^{14}C-residues in the kidney, whereas in mice, maximum residue levels were found in the liver.

In a continuous feeding study, male and female Sprague-Dawley rats and male and female albino mice were placed on diets containing 1, 5, or 25 ppm hex for a maximum of 30 days. Animals were killed at intervals during and after initiation of the study. Excretion patterns were the same for rats and mice, and no sex differences were noted. The major route of excretion was through the feces.

At all dose levels in all animals the kidney, liver, and adipose tissue contained the highest residue levels; and apparent equilibration had been attained after 15 days of feeding, and a positive correlation was observed between the levels of hex in the diet and in the tissues.

It appears that results of this study do not agree closely with the Mehendale study. The Dorough study shows hex to be eliminated from mammals (mice and rats) mainly by the fecal route and with no more than about 15 percent being eliminated in urine. Further, these studies do not indicate any significant amounts of pulmonary elimination of hex or its metabolites. Whitacre (1978) believes that the poor recoveries in feces in the Mehendale study may be the result of volatility of hex or its metabolites before removal for analysis. Losses during sample preparation undoubtedly further complicate the analysis of fecal matter.

EFFECTS

Acute, Subacute, and Chronic Toxicity

The classic studies of hex toxicity to mammals were conducted in the mid 1950's by Treon, et al. (1955). This series of investigations reported on both acute and subacute toxicity of hex to various species of mammals under a variety of exposure regimens. Oral, dermal, and inhalation modes of exposure were included in Treon's experiments. Mammalian toxicity studies subsequent to the 1950's could not be located in the open literature, probably due to the rather low profile of hex relative to other pesticide chemicals. More recent, proprietary studies of the oral and dermal toxicity have now become available. In general, these findings agree remarkably well with those of Treon. It is most unfortunate that no truly long-term (i.e., longer than six months) studies of chronic effects have been conducted. Until data on the potential effects of long-term, chronic exposure (especially carcinogenicity) becomes available, any recommendations regarding environmental criteria must be regarded as tentative.

Acute toxicity of hex was determined by Treon, et al. (1955) by administering dosages of 180, 280, 340, 420, 520, 620, 940, 1,400, and 2,100 mg/kg of hex in peanut oil directly into the stomachs of several groups of rabbits and rats. The data on rabbits indicate that the median lethal oral dose (LD_{50}) administered as described above, lies in the range between 420 and 620 mg/kg of body weight.

Rats showed variation in minimum lethal dose depending on sex. Male rats were somewhat more sensitive in that the lethal dose was

somewhat less than 280 mg/kg body weight, whereas for females the dosage causing death was greater than 280 mg/kg. The LD_{50} for male rats was determined to be 505 mg/kg with 95 percent confidence limits of 387-623 mg/kg.

Kommineni (1978) conducted a study which focused upon gross and histopathological effects at the possible routes of entry and elimination of hex.

In the Kommineni study, a total of 10 female rats were exposed to 0, 50, 100, 150, 200, and 300 mg/kg of hex by gavage. All animals were sacrificed 24 hours post-treatment. The rats were necropsied and lungs, liver, spleen, kidneys, adrenals, heart, stomach, and intestines were saved for histopathology evaluation.

Gross pathology of the rats exposed to 200 and 300 mg/kg revealed brown discoloration around the nostrils and anus of the rats. The urinary bladders of two of the four rats contained brown fluid. Subserosal emphysema of the nonglandular stomach was evident in one animal. On histopathologic examination, the lungs showed atelectasis with moderate thickening of the alveolar walls. The alveolar walls contained moderate numbers of macrophages and neutrophils. Some bronchi contained denuded epithelium. No edema was present in the lungs. Rats receiving lower dosages showed similar, but milder, changes. The stomachs of rats receiving dosages of 200 or 300 mg/kg showed coagulative necrosis of the gastric squamous epithelium. The submucosa of the nonglandular part of the stomach showed mild neutrophilic infiltration. The supporting structures of the stomach (submucosa, submuscularis, muscularis) showed moderate edema. Epithelium of the glandular part of the

stomach showed no treatment-related changes. Animals receiving lower doses showed similar changes in the stomach. Ulcers of the nonglandular portion of the stomach were seen in several of the animals. At all dosages, the other organs were unremarkable.

The author commented that these morphological changes indicate that hex is absorbed through the squamous epithelium of the nonglandular part of the stomach and that the major route of elimination of hex is through the lungs.

The International Research and Development Corporation (IRDC, 1972) conducted similar studies of the acute oral toxicity of hex. Twenty-five albino rats of each sex were given hex dissolved in corn oil at dosages of 315, 500, 794, 1,250, and 1,984 mg/kg. Five rats of each sex were used at each dosage level. An LD_{50} of 530 mg/kg was determined for female rats and 630 for male rats. The combined oral LD_{50} for both sexes was determined to be 584 mg/kg. Note that this is the reverse of the sex difference reported by Treon, et al. (1955). Naishstein and Lisovskaya (1965) reported a LD_{50} of 600 mg/kg for white rats. This value is comparable to the upper part of the range (420-620 mg/kg) reported by Treon, et al. (1955). Thus, the true LD_{50} is probably about 600 mg/kg.

In this series of experiments, 93.3 percent hexachlorocyclopentadiene was applied to the intact skin of rabbits using the technique of Draize et al., described by Treon et al. (1955). It was determined that the lethal dosage lies between 430 and 630 mg/kg body weight. Such a finding is notable in that hex appears to be just as toxic via dermal application as by ingestion.

Kommineni (1978), painted four male guinea pigs on the skin (site unspecified) with hex at dosages of 0, 300, 600, and 1,200 mg/kg and sacrificed 24 hours after the exposure. All animals were necropsied and the lungs, liver, pancreas, kidneys, adrenals, urinary bladder, heart, skin, stomach, and intestines were saved for histopathologic evaluation.

On gross pathology, subcutaneous edema was seen extending from the inguinal area to the sternum. At the lowest dosage, the lungs were highly expanded and showed rib impressions on the parietal surface. Similar but more severe changes were seen in the animal receiving 600 mg/kg. The animal painted with 1,200 mg/kg expired prior to sacrifice; the trachea was filled with frothy fluid. Histopathologic examination of the lungs revealed atelectasis with thickened alveolar walls containing moderate numbers of macrophages and neutrophils. Intense congestion of all pulmonary blood vessels and occasional alveolar edema was seen in the animal receiving the 1,200 mg/kg dose. In the skin, moderate to marked edema disrupted the collagen bundles. Focal pockets of neutrophils were seen in the edematous dermis. Edema extended throughout the thickness of the adipose tissue layer. One animal showed partial thrombosis of medium size veins situated deep in the dermis. The skin appendages were normal.

More recently, the irritant properties of hex were examined in a study conducted by IRDC (1972). These tests were commissioned by Velsicol Chemical Corporation in accordance with the regulations of the Federal Hazardous Substances Act.

IRDC (1972) reported the results of an investigation of acute dermal toxicity of hex to rabbits. Four male and four female New Zealand White rabbits were used in this test. The hair was removed from the back of each rabbit with electric clippers. Two male and two female rabbits were used at each of two dosage levels. The test compound was applied in a single administration to the back of each rabbit at a dosage of 200 or 2,000 mg/kg body weight. The area of application was wrapped with a gauze bandage and occluded with Saran Wrap. Twenty-four hours later, the bandages were removed and the backs were washed with water. The rabbits were observed for mortality for a period of 14 days.

All of the animals which received 2,000 mg/kg dosage died within 24 hours after application of the compound. At the 200 mg/kg dosage, both male rabbits died but both female rabbits survived although they both exhibited weight loss over the 14-day period. The male rabbits that died showed weight loss also. In addition, cachexia, marked dermal irritation, and hypoactivity was observed. Skin at the site of application turned purple within a few hours after hex application. Based on these results, hex was concluded to be "a highly toxic material by the dermal route of exposure" in accordance with the criteria established under the Federal Hazardous Substances Act.

Treon, et al. (1955) exposed various animal species to vapors formed by bubbling a stream of air through liquid hex contained in a bubbling tower. This air was then mixed with clean air to achieve the desired concentration. The stream of air, conditioned with respect to temperature, dust content, and humidity, was then passed

into a plywood exposure chamber in which the test animals were confined. A series of hex concentrations in the air in the exposure chamber were used; these varied from 0.15 to 73.6 ppm. Test species were guinea pigs, rats, mice, and rabbits.

The authors reported that hex vapor was very toxic to all four species of animals. Exposure to the concentration of 13.0 ppm (an intermediate level in this experiment) for 15 minutes produced fatalities in all species except guinea pigs. Of the four species, rabbits appeared to be the most susceptible. Mice, rats, and guinea pigs followed in order of decreasing susceptibility. Table 1 depicts results of the inhalation experiments. The values tabulated correspond to the concentration in ppm which: (1) permitted all animals to survive; (2) killed 50 percent of the animals; and (3) killed 100 percent of the animals.

Animals of the following species died regularly when exposed to hex vapors at the following concentrations and durations: rabbits - 1.5 ppm for seven hours; mice - 1.4 ppm for two 7-hour periods; rats - 1.0 ppm for five 7-hour periods or 3.2 ppm for two 7-hour periods; and guinea pigs - 3.2 ppm for two 7-hour periods.

IRDC (1972) also reported the results of acute inhalation experiments in rats. The test animals were exposed to atmospheric concentrations of approximately 176.2 and 17,624 ppm of the test compound for four hours. Ten rats were tested at each dosage level. Due to the extremely high dosages employed, little information could be derived from the study. No justification of the choice of dosages was given. All of the animals receiving the test compound at either exposure level died within 48 hours. All rats

TABLE 1

Dose Response Data: Inhalation of Hex Vapors*

Species of Animal	Fatalities, Percent	Hex Concentration (in ppm) Lethal to % of Test Animals Indicated		
		1-Hour Exposure	3½-Hour Exposure	7-Hour Exposure
Guinea pigs. . .	0	7.2	3.1	1.5
	50	13.8	7.1	3.2
	100	20.0[a]	12.4	6.7
Rats. . .	0	3.1	1.4	1.5[b]
	50	7.2	3.1	3.2[c]
	100	20.0[a]	7.1	6.7
Mice. . .	0	1.4	1.4[d]	--
	40	7.2	3.1[e]	1.5[e]
	100	13.8	7.1	3.2
Rabbits. . .	0	1.4	--	--
	67	3.1	6.4	--
	100	7.2	7.1	7.5

*Source: Treon, et al. 1955

[a]Duration of exposure was 1.25 hours

[b]25 percent of group died

[c]75 percent of group died

[d]20 percent of group died

[e]80 percent of group died

at the 17,624 ppm dosage level died during the 4-hour exposure period. At the 176.2 ppm atmospheric concentration, one rat died during the exposure period, eight more were dead within 24 hours, and the remaining rat died on the second day of observation.

Signs seen during the exposure period included eye squint, dyspnea, cyanosis, salivation, lacrimation, and nasal discharge. Gross necropsy showed gray coloration of the skin, severe hemorrhage of the lungs, and hydrothorax among rats exposed to 17,624 ppm. Rats exposed to 176.2 ppm revealed congestion of the lungs in all cases.

Based on these results, the investigators concluded that hex is highly toxic material by the inhalation route of administration. Table 2 summarizes the results of acute toxicity studies of hex.

To date, there has not been a satisfactory study of subacute or chronic oral toxicity of hex. One portion of the Treon, et al. (1955) study attempted to examine subacute/chronic oral toxicity but reported that dosages of 180-2,100 mg/kg were fatal within such a short period of time that the investigators were unable to establish an oral dosage which could be tolerated without mortality over an extended period. Similarly, Naishstein and Lisovskaya (1965) reported that oral administration as little as 20 mg/kg for six months was fatal to 20 percent of white rats.

Treon, et al. (1955) examined effects of sublethal concentrations of hex applied to the skin of rabbits and monkeys. In rabbits, dosages as low as 250 mg/kg induced extreme irritation, purplish-black discoloration of the skin and subcutaneous edema. Although the skin lesions healed eventually, damage to the skin in

TABLE 2

Acute Toxicity of Hexachlorocyclopentadiene
By Various Modes of Exposure*

Oral administration[a]		
	Animal	LD_{50}
	Rabbits	420-620 mg/kg
	Rats	505 mg/kg
	Rats[d]	
	Females	530 mg/kg
	Males	630 mg/kg
		Minimum Lethal Dose
	Rats	
	Males	280 mg/kg
	Females	250 mg/kg

Dermal application[b]		
		LD_{50}
	Rabbits	430-630 mg/kg

Inhalation[c]		
	LD_{50} - (dosage expressed as vapor concentration, ppm)	
	Guinea pig	13.8 ppm
	Rats	7.2 ppm

*Source: Treon, et al. 1955

[a]Hex dissolved in peanut oil, administered by gavage

[b]93.3 percent hex solution in Ultrasene, applied to intact skin for 24 hours

[c]LD_{50}s based on 1-hour vapor exposure

[d]Based on data reported by International Research and Development Corp. (1972). Hex dissolved in corn oil

the area of application persisted for many days and the damage varied in severity and extent with the amount (dosage) of the material applied.

A slightly different procedure was employed in the cutaneous exposures of the monkeys. In this case, a series of hex concentrations (0.001, 0.01, 0.1, 1.0, and 10.0 percent) dissolved in Ultrasene were applied to five sites of the abdominal skin. Dosage of each of the solutions was 0.01 ml. No irritation or other changes were noted; however, when 0.05 ml of the 10 percent solution was applied to the back of a monkey for three consecutive days, the skin became severely irritated and necrotic. Subsequent experiments used more concentrated solutions (20, 40, 60, and 90 percent) which were applied (dosage of 0.05 ml) on separate areas of the monkeys' backs. At all concentrations there was discoloration of the skin, ranging from very light to dark tan as the concentration increased. The discoloration was followed by swelling which varied from slight to severe, again depending on concentration. The highest concentration caused cracking, oozing, and serious discharge from the treated areas; intermediate concentrations produced hardening and swelling of the skin.

In guinea pigs, application of solutions containing 0.01, 0.10, 1.0, and 10 percent hex caused no alterations of the skin, but more concentrated solutions (40, 60, and 90 percent) resulted in discoloration, hardening, and necrosis of the skin at the application site. Based on these tests, it appears that the threshold concentration at which hex in Ultrasene induces irritation of the

intact skin lies between 10 and 40 percent for both monkeys and guinea pigs.

Hex was tested for eye irritancy by instilling 0.1 ml of the "test compound" (which was presumably undiluted liquid hex) into the eyes of New Zealand White rabbits (IRDC, 1972). The test material was placed into the conjunctival sac of the right eye of each rabbit; the left eye served as an untreated control. Corneal damage was evaluated by instillation of sodium fluorescein into the eye, followed by examination of the corneal surface for evidence of damage under ultraviolet light. A graded scale was used to quantify the extent and severity of damage. The eyes of the rabbits were checked for corneal lesions at intervals (at 1, 24, 48, and 72 hours post-exposure and at 7, 14, and 21 days post-exposure). Examinations at 14 and 21 days were precluded by the deaths of all of the rabbits on or before the ninth day of the observation period. IRDC investigators attributed the deaths to the effects of the test compound, but unfortunately did not conduct postmortem examinations to rule out other possible causes of death.

Based on the severity of the ocular lesions produced in the rabbits, hex was concluded to be "an extreme irritant and probable corrosive substance" in the 5-minute test and "an extreme irritant and corrosive substance" in the 24-hour wash test (IRDC, 1972). These classifications are set in accordance with standards set under The Federal Hazardous Substances Act, specifically Part 191, Hazardous Substances Test for Eye Irritants, Food and Drug Administration.

When mice, rats, rabbits, and guinea pigs were exposed to 0.34 ppm in air for 7 hours a day for 5 days per week, none of the mice or rats survived more than 20 such exposures (Treon, et al. 1955). Two-thirds of the rabbits had died by the end of the twenty-fifth period; however, the guinea pigs survived through 30 periods. At 0.15 ppm, some animals from all four species survived 150, 7-hour exposures over a period of 216 days. Eight percent of the mice did not survive the prolonged intermittent exposure. Details of these findings are discussed under the heading "chronic toxicity."

In the Treon, et al. (1955) study, rabbits and rats given various dosages of hex ranging from 180-2,100 mg/kg tended not to survive long enough at these dosages to provide acceptable data on chronic oral toxicity. Consequently, these investigators were unable to establish an oral dosage which could be tolerated (e.g., without mortality) over an extended period of time.

Studies in the Soviet Union reported by Naishstein and Lisovskaya (1965) appear to provide the only source of information on the effects of long-term, low-dose exposure to hex. Daily administration of 1/30 of the median lethal dose (20 mg/kg) for six months killed only 2 animals out of 10, even though the cumulative dose received was 1.5 times the acute LD_{100}, and six times the LD_{50}. Although some changes were noted in the weight coefficients of internal organs of the animals, the authors judged the cumulative effects of hex to be weak. No observations of neoplasms or other abnormalities were reported.

Undiluted 93.3 percent hex solution in concentrations of 430 mg/kg, 610 mg/kg, 1,020 mg/kg, 2,130 mg/kg, and 6,130 mg/kg, when applied to the skin of rabbits, was frequently fatal within a few hours. Six rabbits who survived for 7-21 days after application of hex were killed and autopsied. Degenerative changes were seen in the brain, liver, kidneys, and adrenal glands of these animals in addition to chronic skin inflammation, acanthosis, hyperkeratosis, and epilation. Visceral lesions due to dermal hex application reported by Treon, et al. (1955) are described in the section on toxic symptoms and pathological effects.

Naishstein and Lisovskaya (1965) also investigated the effects of multiple, low-dose dermal exposures to hex. These experiments consisted of applying 0.5-0.6 ml of a concentration of 20 ppm hex in aqueous solution to the shaved skin of rabbits daily for a period of 10 days. No differences were detected between the skin of the experimental animals and that of the controls.

Treon, et al. (1955) reported that dosages of less than 10 percent hex appeared to be tolerated without irritative effects in monkeys and probably also in guinea pigs. Unfortunately, neither investigation continued the low-dose regimen for a sufficient period to observe chronic effects.

Treon, et al. (1955) exposed guinea pigs to hex vapors at a concentration of 0.34 ppm hex for seven hours per day, five days a week. All of them survived until they reached 30 periods of exposure in six weeks. Rats and mice exposed to this concentration survived only five periods of exposure; however, survival of the

rabbits was intermediate; two-thirds had died before the end of the fifth week (25 exposure periods).

A lower concentration, 0.15 ppm hex, was tolerated by guinea pigs, rabbits, and rats throughout 150, 7-hour periods of exposure extending over a period of approximately seven months. Four of five mice died within this period. Although guinea pigs, rabbits, and rats appeared to grow normally during this period, slight degenerative changes were observed in the livers and kidneys of these animals. These changes are discussed in the following section.

Rats and rabbits exposed to hex in the Treon, et al. (1955) acute toxicity study exhibited diarrhea, lethargy, and retarded respiration. The odor of hex could also be detected in the feces of these animals and on their bodies, presumably from fecal contamination. Rabbits which died following exposure to moderately high doses of hex (180-2,100 mg/kg hex in corn oil) showed diffuse degenerative changes in the epithelium of the renal tubules. As in the study of Kommineni (1978), the lungs of these animals were congested and edematous. The same types of degenerative changes were also noted in the rats. In addition, some of the rats showed acute necrotic gastritis. Animals which survived the oral tests and were later sacrificed exhibited residual degenerative changes of the type described above, suggesting that the pathological changes are persistent. The severity of the lesions was diminished however, with increasing length of the post-exposure survival interval.

A 90-day subacute oral toxicity study in rats conducted for Hooker Chemical and Plastics Corporation by Industrial Bio-Test

Laboratories (IBT) was reported by Equitable Environmental Health (1976) but the data from this study were not made available for review.

Naishstein and Lisovskaya (1965) reported results of a chronic oral toxicity experiment on 90 white rats. For a period of six months rats were given daily peroral doses of 0.002, 0.0002, and 0.00002 mg/kg (0.04, 0.004, and 0.0004 mg/l) in aqueous solution. The first dose was 30 times greater than the threshold concentration with respect to aftertaste and smell (0.0013 mg/l); the second dose corresponded to the practical limit of detection by smell, and the third dose was 10 percent of the second. No deviations were observed in the behavior of the rats or in their weights throughout the 6-month experimental period. Likewise, no significant changes were seen in hemoglobin, red blood cells, white blood cells, or peripheral reticulocyte counts in the experimental groups as opposed to the controls. In animals receiving the highest dose, 0.002 mg/kg, neutropenia and a tendency toward lymphocytosis were reported. The peripheral blood of animals receiving the two lower dosages did not show any alterations relative to controls. The authors concluded that daily peroral administration of doses of 0.0002 and 0.00002 mg/kg (0.004 and 0.0004 mg/l in aqueous solution) caused no changes in peripheral blood cells, ascorbic acid content, conditioned reflexes, or histologic structure of the organs. Based on these tests and the threshold level for organoleptic noxious effects (smell and aftertaste in water), Naishstein and Lisovskaya (1965) recommended a maximum permissible concentration of 0.001 mg/l hex in water.

Treon, et al. (1955) showed that application of very low dosages of hex (0.25 mg/kg) to the skin of rabbits was extremely irritating and induced local discoloration and edema. The skin became hard, encrusted, and fissured several days after application. The extent of the local damage varied directly with the size of the dose applied. At autopsy rabbits exhibited visceral lesions similar in appearance to those seen after oral administration of hex. Again, diffuse degenerative changes were seen in the brain, heart, adrenals, liver cells, and kidney tubules. Pulmonary hyperemia and edema were also noticed. Animals killed 7-21 days post-application of the compound showed evidence of the same type of degenerative changes.

Monkeys dosed with various concentrations of hex in solution exhibited discoloration of the skin which increased directly as the concentration of hex applied increased. Swelling, oozing, and encrustation similar to that described above for rabbits were seen. Healing eventually took place, but scarring and hair loss in the area of application appeared to be permanent (Treon, et al. 1955).

Industrial Bio-Test Laboratories conducted a 28-day subacute dermal toxicity study using albino rabbits. The study was reported by Equitable Environmental Health (1976) but the data were not made available for review.

Rats, rabbits, guinea pigs, and mice exposed to vapors of hex showed signs of extreme irritation of the eyes and mucous membranes (Treon, et al. 1955). At very high concentrations (46.5 ppm) animals responded by rubbing their noses with their forefeet, closing

their eyes and retracting their heads. This behavior was accompanied by sneezing, tearing, and irregular breathing. In less than 30-60 minutes the animals were gasping for breath.

Lower concentration of hex vapor (12.4 and 13.8 ppm) produced similar irritation of the mucous membranes, although somewhat milder in degree. The same symptoms were even seen at the low dosages (1.0 and 1.6 ppm), but the symptoms developed over a period of hours rather than minutes. Exposure to very low concentrations (0.33 ppm and 0.15 ppm) resulted in some irritation of the eyelids and increased respiratory rate. In the case of the latter dosage (0.15 ppm), irritation was seen only in the mice, which developed mild respiratory changes (Treon, et al. 1955). Rats which survived the vapor exposure sessions lost weight and many of these animals failed to regain their initial weights as long as six to eight weeks after cessation of the exposures.

At autopsy Treon, et al. (1955) reported degenerative changes similar to those described above (oral and dermal administration experiments) in all species of animals tested. Prolonged intermittent exposure to vapor concentrations as low as 0.15 ppm hex induced slight degenerative changes in the livers and kidneys in all species of animals employed.

Equitable Environmental Health (1976) also reported results from two vapor toxicity studies, an acute test and a 28-day subacute test which were conducted by Industrial Bio-Test Laboratories (IBT) for Hooker Chemical and Plastics Corporation but public review of the test data was not allowed.

Synergism and/or Antagonism

There does not appear to be any information available on synergistic or antagonistic effects between hex and other compounds.

Teratogenicity

International Research and Development Corporation (IRDC, 1978) has recently completed a pilot teratology study using pregnant Charles River (CD) rats. Negative findings with respect to teratogenic effects were reported for oral hex dosages up to 100 mg/kg/day.

The test protocol employed in the pilot teratology study involved administration of various dosages of hex to 30 female Charles River (CD) rats approximately 12 weeks of age. Females were mated with male rats of the same strain. After mating, the females were assigned to six groups, one control and five treatment groups of five rats each. Hex was dissolved in corn oil and administered by gavage from day 6 through day 15 of gestation. Dosage levels of 3, 10, 30, 100, and 300 mg/kg/day were administered to the test groups and the control group was given the vehicle (corn oil) on a comparable regimen of 10 ml/kg/day.

During gestation, the females were observed for clinical signs of toxicity, mortality, and body weight gains. They were then sacrificed on gestation day 20 and the uterine contents examined for viable and nonviable fetuses, early and late resorptions, and total implantations. There were no differences in the four treatment groups given 100 mg/kg/day or less when compared to the control group in terms of number of viable or nonviable fetuses, resorptions, implantations, or corpora lutea. Rats receiving doses of 3 or 10 mg/kg/day showed no treatment-related changes in appearance

or behavior. Rats receiving 30 mg/kg/day or higher showed staining of the anogenital area and reduced body weight gains. The females in the 100 mg/kg/day group had body weight losses during the first three days of treatment and reduced weight gains for the remainder of the study. Survival was 100 percent for all rats given 100 mg/kg/day or less. All rats in the 300 mg/kg/day group were dead by gestation day 10.

Various reproductive parameters examined in the pilot teratology study are shown in Table 3.

Mutagenicity

Hex has been tested for mutagenicity and reported nonmutagenic in both short-term in vitro mutagenic assays (National Cancer Institute, 1977; Industrial Bio-Test Laboratories, 1977; Litton Bionetics, 1978a) and in a mouse dominant lethal study (Litton Bionetics, 1978b).

The National Cancer Institute (NCI, 1977) reported that preliminary results indicated that hex was nonmutagenic in Escherichia coli K12 (mutation site not specified) in the presence of a mammalian metabolic activation system containing mouse liver microsomes.

Negative results were also reported by Industrial Bio-Test Laboratories (1977) using a test protocol almost identical to the Ames mutagenic assay (Ames, et al. 1975). The tests used four strains of Salmonella typhimurium with and without metabolic activation. Hex was dissolved in acetone and added to the microbial assay plates in dosages from 10-5,000 µg/10 µl. Concentrations greater than 10 µg/10 µl produced a bactericidal effect in three of

TABLE 3

Pilot Teratology Study in Rats: Caesarean Section
Data For Individual Females*

Dosage Level	Viable Fetuses	Non-viable Fetuses	Late Re-sorptions	Early Re-sorptions	Post Implantation Loss	Implantations	Corpora Lutea
Control:							
Total	65	0	0	4	4	69	80
Mean	13.0	0.0	0.0	0.8	0.8	13.8	16.0
3 mg/kg/day:							
Total	76	0	0	1	1	77	82
Mean	15.2	0.0	0.0	0.2	0.2	15.4	16.4
10 mg/kg/day:							
Total	68	0	0	3	3	71	73
Mean	13.6	0.0	0.0	0.6	0.6	14.2	14.6
30 mg/kg/day:							
Total	56	0	0	1	1	57	65
Mean	11.2	0.0	0.0	0.2	0.2	11.4	13.0
100 mg/kg/day:							
Total	68	0	0	2	2	70	70
Mean	13.6	0.0	0.0	0.4	0.4	14.0	14.0
300 mg/kg/day:							
Dam Number							
73758	Died, gestation day 9 - gravid						
77324	Died, gestation day 10 - gravid						
77333	Died, gestation day 10 - gravid						
77417	Died, gestation day 10 - gravid						
77445	Died, gestation day 10 - gravid						

*International Research and Development Corp., 1978

the four strains tested; a possible lethal effect occurred at 2,500 µg/10 µl or greater in the fourth strain. A repressive effect was noted in three of the four strains at concentrations below 10 µg/10 µl. Volatilate (volatile vapors) of hex was also tested on one strain using the vapor from hex concentrations of up to 2,500 µg/10 µl and exposure times of up to two hours. Results from two successive assays in the absence of rat liver enzymes (hex concentrations 10, 25, 50, 75, and 100 µg/10 µl) were negative in all four tester strains. Two assays using the same dosages in the presence of rat liver microsomes were reported nonmutagenic; similarly, negative results were obtained for the hex effusate as well. The investigators expressed concern over the repressive effect of hex on the test bacteria, stating "It appears that hex is probably nonmutagenic and that some toxic effect prevailed with respect to the tester strains required for this assay. Analysis of variance and multiple comparison of the data confirms this observation."

Litton Bionetics (1978a) conducted a mouse lymphoma cell assay in order to evaluate the capability of hex in inducing specific locus forward mutation. The indicator cells used in the assay were Fischer mouse lymphoma cells derived from cell line L5178Y. These cells are heterozygous for a specific autosomal mutation at the TK locus and are bromodeoxyuridine (BUdR) sensitive. Scoring for mutation is based on selecting cells which have undergone forward mutation from a TK+/- to a TK-/- genotype by cloning them in soft agar with BUdR. Cells were maintained in Fischer's medium for leukemic mouse cells with 10 percent horse serum and sodium pyruvate. The dosages used in the test were predetermined by exposing the

cells to a wide range of hex concentrations and measuring the reduction of growth potential following a 4-hour exposure at each dose. The maximum dose selected was that which produced a 50 percent reduction in growth. The actual hex dosages employed were:

0.00040 µl/ml;

0.00048 µl/ml;

0.00056 µl/ml;

0.00064 µl/ml; and

0.00125 µl/ml

in the activated series (mouse liver microsomes were added to the growth medium). A nonactivated series using somewhat lower dosages was included also.

Both negative and positive controls were used; the negative control for both series was the solvent dimethylsulfoxide (DMSO), whereas ethyl methanesulfonate (EMS) and dimethylnitrosamine (EMN) were used as positive controls in the nonactivated and activated systems, respectively. Hex was added to the cells in the growth medium for four hours. The cells were then washed and allowed to express in the growth medium for three days. After the expression period, results were evaluated by counting the TK-/- mutants after cloning the cells in a selection medium (soft agar with BUdR).

Hex dissolved in DMSO was evaluated over the concentration range of 0.0000025 µl/ml to 0.00125 µl/ml. Considerable toxicity occurred at concentrations greater than this and the extent varied according to the presence of the mouse liver activation system as shown in Table 4. No cells treated with hex (at the concentrations shown) survived in the non-activated system.

TABLE 4

Summary of Mouse Lymphoma ($L5_{178X}$) Results*

A. Name or code designation of the test compound: Hexachloropentadiene
B. Solvent: DMSO
C. Test date: 12/18/77
NOTE: Concentrations are given in microliters (ul) or micrograms (ug) or nanoliters (nl) per milliliter.

TEST	S-9 Source	S-9 Tissue	Daily Counts (Cells/ml x 10E5) 1	2	3	Relative Suspension Growth (% of control)	Total Mutant Clones	Total Viable Clones	Relative Cloning Efficiency (% of control)	Percent Relative Growth**	Mutant Frequency*** (X 10E-6)
NONACTIVATION											
Solvent control	---	---	16.8	10.2	13.8	100.0	48.0	257.0	100.0	100.0	18.7
Negative control	---	---	13.2	12.0	15.0	100.5	48.0	234.0	91.1	91.5	20.5
EMS .5ul/ml	---	---	9.0	9.2	11.8	41.3	597.0	89.0	34.6	14.3	670.8
ACTIVATION											
Solvent control	mouse	liver	15.2	9.6	13.2	100.0	55.0	281.0	100.0	100.0	19.6
Negative control	mouse	liver	14.2	13.0	10.6	101.6	39.0	293.0	104.3	105.9	13.3
DMN .5ul/ml	mouse	liver	7.2	7.6	8.2	23.3	322.0	55.0	19.6	4.6	585.5
Test compound											
0.00002 ul/ml	mouse	liver	16.8	9.0	10.6	83.2	99.0	288.0	102.5	85.3	34.4
0.00004 ul/ml	mouse	liver	13.0	12.4	9.6	80.3	50.0	269.0	95.7	76.9	18.6
0.00008 ul/ml	mouse	liver	12.4	9.8	16.2	102.2	55.0	194.0	69.0	70.6	28.4
0.00016 ul/ml	mouse	liver	13.6	13.8	7.4	72.1	45.0	359.0	127.8	92.1	12.5
0.00032 ul/ml	mouse	liver	18.2	9.0	10.0	85.0	38.0	309.0	110.0	93.5	12.3

* Source: Litton Bionetics, 1978a
** (Relative suspension growth X relative cloning efficiency) / 100
***(Mutant clones/viable clones) X 10E-6

Hexachlorocyclopentadiene did not induce forward mutation in L5178Y cells. The data presented in Table 4 show the concentrations of the test compound employed, the number of mutant clones obtained, surviving populations after the expression period, and calculated mutation frequencies. No dose-related trends in either absolute number of mutants or mutant frequencies were observed, and at no level did any of the test parameters increase significantly over the spontaneous level. Consequently, hex was considered to be nonmutagenic under the conditions of this assay.

The mutagenic properties of hex were also evaluated in a dominant lethal study of mice (Litton Bionetics, 1978b). The dominant lethal assay provides a means of determining whether a compound is capable of inducing damage in the germ cells of treated male mice. Dominant lethality is manifested in various forms of fetal wastage, both pre-and post-implantation. Positive dominant lethal assays indicate that a compound is able to reach the developing germ cells. Chromosome aberrations including breaks, rearrangements, and deletions as well as ploidy changes and nondisjunction are believed to produce positive results on this test. Since substances capable of producing gross chromosomal lesions are probably capable of producing more subtle balanced lesions or specific locus mutations, the test also provides suggestive evidence of nonlethal mutations transmissible to future generations as well.

Litton Bionetics reported negative results, that is, there was no evidence of significant dominant lethal activity by hex in mice. The test protocol called for the assignment of ten random bred male mice to one of five groups. Three test groups received hex at dos-

ages of 1.0 mg/kg, 0.3 mg/kg, and 0.1 mg/kg, respectively. These dosages were determined by deriving an LD_{50} level (1.0 mg/kg) and taking one-third and one-tenth of that dose. A fourth group received only the solvent and the fifth group served as a positive control. Hex was administered to the three experimental groups and to the solvent control group by gavage for five consecutive days. The positive control group received a known mutagen, triethylenemelamine (TEM) in a single intraperitoneal injection. Two days following treatment, each male was caged with two unexposed virgin females. At the end of seven days, these females were removed and replaced by two unexposed virgin females. This mating cycle was continued for seven weeks. Each pair of female mice was killed two weeks after mating and necropsied. Their uterine contents were examined for dead and living fetuses, resorption sites, and total implantations. All test parameters [fertility index, average implantations per pregnancy, average resorptions (dead implants) per pregnancy, proportion of females with one or more dead implantations, proportion of females with two or more dead implantations, and the ratio of dead implantations to total implantations] were within normal limits based on historical and concurrent control levels for this test. Thus, there was no evidence of dominant lethal activity in any of the hex treated groups. The positive control group, however, did show the expected dominant lethal activity.

Carcinogenicity

Various types of evidence may be used in evaluating the possible carcinogenic activity of a substance. In order of prefer-

ence, these include: (1) human data; (2) animal data; (3) short-term (*in vitro*) tests; (4) metabolic pattern; and (5) structure-activity relationships. This section summarizes what is known about each of the above.

No epidemiologic studies or case reports examining the relationships between exposure to hex and cancer incidences could be found in the literature. As indicated previously, Hooker Chemicals and Plastics Corporation reports that an in-house study of the mortality patterns of hex-exposed workers is now underway; however, the study is far from being completed (Zavon, 1978, personal communication). Other in-house studies of workers employed in the manufacture of pesticides (including hex) are reportedly being conducted by Velsicol Chemical Corporation. We were unable to obtain any further information on the current status or findings of these studies.

The National Cancer Institute concluded that toxicologic studies of hex in animals have not been adequate for evaluation of carcinogenicity (NCI, 1977). Chronic toxicity studies as reported in the Toxic Symptoms and Pathologic Effects section, were based on too few animals in some cases and/or the duration of the experiments was too short for appropriate evaluation of chronic effects, including carcinogenicity.

Only one short term *in vitro* test of hex for carcinogenic activity could be identified.

Litton Bionetics (1977) reported the results of a test to determine whether hex could induce malignant transformation in BALB/3T3 cells *in vitro*. The cells and methodology of the test

were those of Dr. T. Kakunaga (1973), described elsewhere. The basic rationale of the test and its validity as an indicator of carcinogenic activity were described by the investigators as follows:

> The endpoint of carcinogenic activity is determined by the presence of fibroblastic-like colonies which are altered morphologically in comparison to the cells observed in normal cultures. These (transformed) cells grow in criss-cross, randomly oriented fashion with overlapping at the periphery of the colony. The colony exhibits dense piling up of cells. On staining, the foci are deeply stained and the cells are basophilic in character and variable in size. These changes are not observed in normal cultures, which stain uniformly.

Cell cultures with very little or no spontaneous transformation are maintained for use in these tests. The data generated at each dose level of the test material are analyzed using the t statistic. A significant set of data for any dose level may be sufficient to indicate a positive response. Because this assay is still nonroutine, and definitive criteria for evaluation have yet to be developed, scientific judgment and expert consultation are needed for appropriate interpretation of results.

The BALB/3T3 cells used in the test were grown in Eagle's minimal essential medium (EMEM) supplemented by 10 percent fetal calf serum. Cultures were passaged weekly in 60 mm culture dishes. Approximately 10,000 cells were seeded into 50 ml sterile tissue culture flasks and incubated in EMEM to permit attachment. After the cells were attached, the control and test compounds were added to the plates. Dosages of 0.00001 µl/ml; 0.00002 µl/ml; 0.000039 µl/ml; 0.000078 µl/ml; and 0.000156 µl/ml of hex were employed. The maximal dosage, 0.000156 µl/ml, was determined by selecting

from preliminary cytotoxicity tests the maximum dosage which permitted survival of at least 80 percent of the cells. 3-Methylcholanthrene at 5 µg/ml was used as a positive control and the test compound solvent was used as a negative control. Ten replicates per dose level were prepared and chemical exposure was maintained for 48 hours. Plates were then washed free of the compound and replenished with fresh growth medium. The plates were then incubated for an additional three to four weeks with twice weekly medium changes. Cell integrity was monitored by daily observations. Cells were separated from the medium, washed with saline, and stained. They were examined for stained foci; all potential foci were examined microscopically. Results were presented as the number of foci per set of replicate plates at each dosage level.

The test material was quite toxic to cells as indicated in the preliminary range-finding tests. No significant carcinogenic activity for hex was reported under the conditions of this test. A low level of spontaneous transformation was observed on all of the plates. Only the 3-methylcholanthrene treated plates showed a significantly higher number of transformed foci than the negative control.

It should be noted that in this and other cell culture tests, extremely low dosages of hex were used. Because hex is relatively toxic to cells in culture and test protocols normally require a high survival rate, the applicability of test results to environmental conditions is unclear. Taken together, however, the mutagenicity and carcinogenicity tests conducted by Litton (1977, 1978a) suggest that toxicity, rather than chronic effects, is per-

haps the critical effect of hex, even at very low dosages. Extremely poor survival has also been problematic in several subchronic tests of hex in mammalian species.

A very recent study involving chronic dietary exposure of rats to hexachlorobutadiene also provides some insight into the relationship between direct toxic effects and chronic effects (i.e., carcinogenesis) in this related compound (Kociba, et al. 1977).

Male and female Sprague-Dawley rats were maintained on diets supplying 20, 2.0, 0.2, or 0 mg/kg/day of hexachlorobutadiene (HCBD) for up to two years. Rats ingesting 0.2 mg/kg/day had no discernible ill effects that could be attributed to this dose level. Ingestion of the intermediate dose level of 2.0 mg/kg/day caused some degree of toxicity, affecting primarily the kidney in which increased renal tubular epithelial hyperplasia was noted. Urinary excretion of coproporphyrin was also increased at this dose level. Ingestion of the highest dose level of 20 mg/kg/day caused a greater degree of toxicity. Effects included decreased body weight gain and length of survival, increased urinary excretion of coproporphyrin, increased weights of kidneys, and renal tubular adenomas and adenocarcinomas, some of which metastasized to the lung. In this study irreversible toxicological effects, such as the development of neoplasms, occurred only at a dose level which caused significant tissue injury and other manifestations of toxicity. No neoplasms resulted with dose levels which caused no injury or only mild, reversible injury.

Little information is available on the metabolism of hex. Although at least four metabolites were found in the Mehendale (1977)

study, at the time of this writing they had not been identified. Thus, the metabolic pathway is uncertain.

As far as structure/activity relationships are concerned, the National Cancer Institute (1977) speculated that as a cyclopentene vinyl halide, hex potentially may be metabolized to an electrophile. In addition, hex is related to the pesticides dieldrin, heptachlor, and chlordane which have been found to induce liver tumors in mice following oral administration (NCI, 1977).

Hex has recently been selected for testing in the National Cancer Institute's test program (NCI, 1977). The reasons given for its selection include: (1) its high potential for exposure (as an industrial intermediate used in the manufacture of pesticides, flame retardants and dyes, pharmaceuticals, resins, and germicides); (2) its suspect chemical structure; and (3) the relative lack of information on the effects of chronic exposure to this compound.

Extremely limited data are available concerning the effects of hex exposure on humans. That which is known about acute human toxicity is based largely upon isolated spills or other accidental incidents involving pesticide workers, laboratory technicians, or others having occupational contact with hex. A recent incident in which approximately 200 sewage treatment plant workers were exposed to acutely toxic levels of hex from the illegal disposal of large quantities of the compound has done much to elucidate the potential health effects of acute human exposures. Due to the accidental and episodic nature of these incidents and the lag time in setting up environmental monitoring equipment in response to the incidents, it

has not been possible to measure environmental concentrations of hex at the exact time workers report symptoms (post-exposure sampling results are sometimes available). Thus, while there is information regarding the range and variety of toxic responses, the exact dose which elicited a given response remains unknown. It is obvious that reliable dose-response estimates require accurate measurement of both dose and response parameters.

Likewise, virtually nothing is known regarding the potential effects resulting from chronic exposure to environmental sources of hex. Potential modes of environmental exposure (e.g., through exposure to contaminated air or water) are uncertain at this time.

According to Hooker's material safety data sheet for hexachlorocyclopentadiene (Hooker Ind. Chem. Div., 1972), the compound is very irritating to the eyes and mucous membranes causing lacrimation, sneezing, and salivation. Repeated contact with the skin can cause blistering burns, and inhalation can cause pulmonary edema. Hex is readily absorbed through the skin. Ingestion can cause nausea, vomiting, diarrhea, lethargy, and retarded respiration. Recommendations for safe use include: (1) good general ventiliation plus local exhaust at points of potential fume emission; (2) respiratory protection of the organic vapor-acid gas canister type and full-face self-contained breathing apparatus for emergencies; (3) elbow-length neoprene gloves; (4) eye protection including chemical safety glasses, plus face shield where appropriate; and (5) protective clothing including full-length clothing fastened at neck and wrist, rubber safety shoes or boots, rubber or other impervious clothing or aprons as needed for splash protection.

According to Treon et al. (1955), a very faint odor of hex was detectable in air by some individuals at concentrations as low as 0.15 ppm which was the lowest concentration employed in their experiments. At approximately twice that concentration (0.33 ppm), a very pronounced, pungent odor was present.

Treon, et al. (1955) observed that headaches developed among laboratory workers following incidental exposure to hex vapor from the respiratory chambers used for their vapor inhalation experiments. The exact concentration of hex escaping into the laboratory from the opening of the respiratory chamber is unknown; however, the chamber was not opened until the contaminated air had been exhausted and the chamber flushed for some time with clean air. Thus, the ambient concentration producing headaches among the laboratory workers was well below the dosages employed in the animal experiments. Because no mention is made of any other irritative symptoms (e.g., lacrimation, etc.), it seems reasonable to speculate that the concentration of hex present was somewhere in the range between 0.15 ppm-1.0 ppm, above the detection threshold but below the level producing acute symptoms of irritation.

Irritant effects are elicited at a vapor concentration greater than that shown to produce chronic toxicity in animals. Thus, Treon et al. (1955) concluded that the irritant effects of hex vapors are not sufficiently pronounced to serve as a warning that a hazardous level of hex vapor is present and/or that hazardous exposure is taking place.

According to Naishstein and Lisovskaya (1965), hex may be detected by taste and smell at very low concentrations in water.

They placed the threshold level for altering the organoleptic qualities of water at 0.0014-0.0010 mg/l.

Epidemiologic Studies

To date, the only well documented incident of the acute toxicity of hex to humans occurred at the Morris Forman Wastewater Treatment Plant (MFWTP) in Louisville, Kentucky. The problem apparently began about the middle of March, 1977, when an unknown chemical, later identified as a mixture of hex and octachlorocyclopentene (Table 5), began entering the Morris Forman sewage treatment facility. An exact date of initial appearance at the plant, and hence, the initial date of worker exposure is unknown. However, unusual odors became evident around March 17, 1977.

The odor gradually intensified over the next two weeks. From March 25-28, an odoriferous, sticky material entered the plant and gummed the barscreens and grit collection systems in the primary treatment area. Attempts to dislodge the material with steam produced a blue gas which permeated the grit removal and sludge handling areas. Workers exposed to this vapor complained of severe irritation of the eyes, nose, throat, and lungs (Carter, 1977b). Approximately 20 workers sought medical treatment for tracheobronchial irritation. These workers were treated in the local emergency room: none were hospitalized (Singal, 1978).

A sample of the material from the Screen and Grit Building was sent to the U.S. EPA Laboratory in Athens, Georgia, for analysis. The primary contaminants in the samples were identified as hexachlorocyclopentadiene (hex) and octachlorocyclopentene (octa). Octa is a waste by-product in the manufacture of hex whose toxicity

TABLE 5

Analysis[1] of a Sludge Sample Obtained in the Screen and Grit Building on April 2, 1977, Morris Forman Wastewater Treatment Plant, Louisville, Kentucky*

Compound[2]	Concentration - % by weight
Octachlorocyclopentene	9
Hexachlorocyclopentadiene	4
Hexachlorobenzene	0.3
Pentachlorobenzene	0.2
Octachloronaphthalene	0.4 (estimated)
Heptachloronaphthalene	0.2 (estimated)
Hexachloronaphthalene	(not quantitated)
Mirex	0.007 (estimated)

*Source: Singal, 1978

[1]Analysis was conducted by the U.S. Food and Drug Administration, Division of Chemical Technology, Chemical Industry Practices Branch

[2]The sample was analyzed using gas chromatography interfaced with mass spectroscopy for positive identification of each compound

is presently unknown. Table 5 shows the results of the analysis. Due to the apparent potential toxicity of hex (and the unknown toxic potential of octa), the sewage treatment plant was evacuated and closed on March 29, 1977. Thereafter, until the partial re-opening in June, 1977, 105 million gallons per day of domestic and industrial wastes were diverted directly to the Ohio River.

Estimates of the extent of contamination indicate that about 60 million gallons (25,000 tons) of hex-contaminated material were present at the Morris Forman plant. Of this, approximately 6 tons of hex and octa were thought to be present in the contaminated waste. U.S. EPA's analysis revealed hex concentrations up to 1,000 ppm in the sewage water at the time of the plant closure. The route of chemical contamination was traced to one large sewer line which passed through several heavily populated areas. Wastewater in this sewer showed hex and octa in concentrations ranging up to 100 ppm. Samples from the sewer showed air concentrations ranging up to 0.4 mg/l for hex and up to 0.03 mg/l of octa. Thus, it was decided to investigate the health of not only the workers at the sewage treatment plant but also residents of the area surrounding the sewer line (Morse, et al. 1978).

A cooperative investigation involving Region IV, U.S. EPA (Surveillance and Analysis Division), Center for Disease Control (CDC), National Institute for Occupational Safety and Health (NIOSH), Jefferson County (Kentucky) Health Department, and the Kentucky State Health Department was initiated.

Information on both aspects of the investigation (i.e., community residents on one hand and exposed workers on the other) is

thus far unpublished but preliminary drafts of reports were made available by Dale Morse, M.D., who headed the initial epidemiologic studies conducted by the Center for Disease Control (Morse, et al. 1978) and by Mitchell Singal of the Hazard Evaluation and Technical Assistance Branch of NIOSH who reported on the follow-up investigations of workers during cleanup operations at the sewage treatment facility (Singal, 1978). Findings from these drafts are reported below; however, they should be regarded as preliminary.

The Center for Disease Control investigation began by identifying all sewage treatment employees who worked at the plant for two or more days during the period from March 14-29, 1977. Health effects evaluations, including mailed questionnaires, physical examination, and blood and urine testing, were conducted appropriately to exposed individuals who agreed to participate. The questionnaire covered demographic information, a detailed work-area history, symptoms and history of chemical poisoning, personal habits, and other sources of chemical exposure. Routine tests were performed on blood and urine specimens. Additional samples were sent to NIOSH laboratories for potential toxic chemical analysis.

Of 193 plant employees who had worked during the latter half of March, questionnaire data were obtained from 145. Seventy-five percent of the questionnaire respondents indicated that they detected an unusual odor at the plant sometime during March. A few individuals reported detecting unusual odors as early as March 1, 1977; the percentage reporting the odor by March 14 was noticeably

increased. From March 15 onward, the percentage of workers who reported noticing the odor steadily increased until the plant was closed on March 29.

A comparison between the time of odor detection and the onset of eye irritation, the most common symptom, showed that irritation developed on the same day in 45 percent of individuals, within 1 to 5 days in 28 percent, and after five days in 21 percent. Only 6 percent of employees reported onset of symptoms prior to noticing an unusual odor at the plant.

Eye irritation, headache, and throat irritation were the most common symptoms, with 59 percent, 45 percent, and 27 percent of employees reporting these symptoms, respectively. Data for these and other symptoms are reported in Table 6. Of 41 workers physically examined, five had signs of eye irritation (tearing and/or redness) and five had signs of skin irritation.

Forty-two persons were interviewed and provided blood and urine samples. This included 24 of 29 (83 percent) of the workers who had been previously evaluated by local physicians, 17 of 164 other plant employees (a 10 percent random sample) as well as one non-employee accidentally exposed to the contaminated sludge.

Abnormalities were found in laboratory analysis of some of the workers (e.g., LDH elevations in 27 percent and proteinuria in 15 percent of those examined). No LDH or urinalysis abnormalities were corroborated on repeat tests run three weeks later by another laboratory. Also, no abnormalities were reported among individuals seen at the local hospital or by the plant physician.

TABLE 6

Symptoms of 145 Plant Employees,
Louisville, Kentucky, March, 1977*

Symptom	Number with Symptom	Percent with Symptom
Eye irritation	86	59
Headache	65	45
Throat irritation	39	27
Nausea	31	21
Skin irritation	29	20
Cough	28	19
Chest pain	28	19
Difficult breathing	23	16
Nervousness	21	14
Abdominal cramps	17	12
Decreased appetite	13	9
Decreased memory	6	4
Increased saliva	6	4

*Source: Morse, et al. 1978

Detailed work area histories on 124 individuals during the highest exposure period showed that "cases" occurred in all areas of the plant. A case was defined as an individual who reported two or more major symptoms (eye irritation and headaches) or one major symptom and two minor ones (sore throat, cough, chest pain, difficulty breathing, skin irritation). Attack rates were significantly higher for individuals who had been exposed to the screen and grit chamber (p .0001) and to the primary settling area (p .02) than for workers not exposed to these areas.

Analysis of data according to employee work areas revealed that symptoms occurred in workers of all job categories and in all work areas. Data for attack rates in employees by main work area are reported in Table 7. Only small differences in case rates appeared by work area although the highest attack rates occurred in workers in the primary treatment area where the level of hex was presumably highest. Attack rates were significantly higher by X^2 (chi-square) test for individuals who had been exposed to the screen and grit chamber ($p = .0001$) and to the primary settling area ($p = .02$) than for workers not exposed to these areas.

The initial investigation demonstrated that 64 of 145 (44 percent) of current employees questioned at the wastewater treatment plant had experienced headache and mucous membrane, skin, and respiratory tract irritation after exposure to airborne hex. Highest attack rates occurred among workers in the primary treatment area where exposure was highest and ventilation poorest. In most cases symptoms were transient, but in some workers, they persisted for several days. This episode clearly demonstrates the volatility

TABLE 7

Attack Rates in Employees by Main Work Area,
Louisville, Kentucky, March, 1977*

Main Work Area	Number of Employees	Number Reporting Symptoms	Percentage of Employees Reporting Symptoms	Percentage of Cases of Those Reporting Symptoms
Primary treatment	19	17	89	59
Throughout plant	71	54	76	48
Vacuum filtration	19	15	79	47
Secondary aeration chamber	14	12	86	42
Administration and laboratory	30	22	73	41
Final effluent pump station	10	5	50	40
Low pressure oxidation	13	10	77	30
Incineration	17	10	50	20
Totals	193	145	75	44

*Source: Morse, et al. 1978

of hex and its potential for having a toxic effect on humans. Results of the follow-up investigation of the sewage treatment plant workers and the community survey are reported below.

After the initial health evaluation survey was completed (April 3, 1977), NIOSH assumed the responsibility for follow-up investigations of the sewage treatment workers exposed during the March, 1977, episode. NIOSH was also responsible for medical monitoring of those involved in the cleanup operations prior to reopening the Morris Forman plant. NIOSH's activities consisted of the following: (1) administering follow-up questionnaires to all plant employees to determine how persistent symptoms had been after the initial chemical exposure in March; (2) review of the medical records of the 90 employees who had seen the plant physician from late March through May 10, 1977; (3) collection of repeat biologic samples on the 23 employees who had shown some abnormality on the testing done by the CDC physicians (March 31-April 2, 1977, tests); (4) biological monitoring of EPA and NIOSH industrial hygienists and environmental technicians exposed to the chemicals in the sewer system during cleanup; and (5) medical monitoring of Morris Forman plant employees who were actively involved in the plant cleanup. Results of each of these aspects of the investigation are reported below.

Usable responses were obtained from 182 individuals on the follow-up questionnaire. The frequency of symptoms among those who completed the questionnaire is shown in Table 8. In decreasing order of frequency, these symptoms included eye irritation, headache, fatigue, chest discomfort, sore throat, cough, nausea, and skin rash. These symptoms were surprisingly persistent. Except

TABLE 8

Symptoms Reported on Follow-up Questionnaire,* Morris Forman Wastewater Treatment Plant, Louisville, Kentucky**

Symptom	% with Symptoms in Last 2 Weeks of March***	Persistence of Symptoms*** (% of those with symptoms)			
		Gone Within 1 Day	Gone Within 1 Week	Gone Within 2 Weeks	Still Present at Time of Survey
Headache	55%	19%	30%	18%	32%
Eye Irritation	62%	36%	23%	16%	15%
Sore Throat	30%	15%	49%	13%	18%
Cough	24%	14%	27%	16%	36%
Chest Discomfort	34%	11%	20%	21%	39%
Skin Irritation	21%	18%	18%	10%	46%
Nausea	22%	18%	23%	18%	25%
Fatigue	34%	8%	16%	24%	45%

* Distributed and collected last 2 weeks of May 1977
Excludes employees actively involved in cleanup, since their symptoms could relate to exposure during cleanup instead of to exposure prior to the plant shutdown.

** Source: Singal, 1978

***Percentages do not quite add to 100% due to some employee confusion about the need to fill in questionnaire completely.

for eye irritation and sore throat, 25-45 percent of those who exhibited symptoms during the last two weeks of March, 1977, still had them six weeks later. Although symptoms occurred in workers in all areas of the plant, maintenance department personnel consistently reported the highest number of symptoms.

A review of medical records of the 90 workers examined by the plant physician (mid-March to May 10, 1977) revealed symptom reports similar to those reported on the NIOSH and CDC questionnaires. Fatigue, headache, and mucous membrane irritation were the predominant complaints; respiratory and skin problems were also reported. Seven of the 90 workers reported transient memory loss ranging from a few minutes to a few days. These are believed to represent a transient state of confusion, rather than true amnesia (Singal, 1978). Although several workers reported neurologic symptoms, the plant physician found no one with any objective neurologic signs. Seven persons had rash on exposed areas of face and arms. Respiratory tract symptoms, cough, and chest discomfort were commonly reported. Twenty-eight persons, including those with respiratory symptoms, received chest x-rays. Essentially all of the x-rays were normal. Sixteen persons received blood gas determinations, none of which showed an elevated pCO_2 or a pO_2 below 70 mm Hg. Pulmonary function tests were done on 22 individuals but no significant pattern of abnormalities was seen. Cholinesterase levels on 27 workers were normal. Several workers had elevated liver function tests; these were mainly minor elevations of lactic dehydrogenase (LDH) and alkaline phosphatase which are difficult to interpret. More specific liver function tests such as serum gluta-

mic oxalacetic transminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were elevated in three persons. Six elevations of bilirubin, two elevations of serum creatinine, and six mild proteinurias were detected. Unfortunately, the specimens were analyzed by at least three different laboratories and comparison/interpretation of these results is uncertain. Attempts to develop a technique to isolate and identify concentrations of hex in specimens of blood or urine at the time of the investigation were unsuccessful (Morse, et al. 1978).

Biological monitoring of NIOSH and U.S. EPA personnel who were actively involved in the cleanup effort showed no significant abnormalities.

Repeat laboratory tests were done on 20 of the 23 sewage treatment plant workers who had abnormalities on the blood and/or urine tests at the time of plant shutdown. Three of these people continued to have persistent abnormalities in liver function tests on one or more occasions but there were no persistent urinary abnormalities.

Exposure levels of the cleanup crew were monitored by taking samples of breathing zone concentrations (inside masks) of hex and octa. These values are reported in Tables 9 and 10.

Biological monitoring of the cleanup crew was also conducted by NIOSH. Due to continuous turnover of crew members, it was not possible to obtain pre-exposure baseline studies on more than 54 percent of the workers. Symptoms reported by crew members were similar to those reported on the NIOSH and CDC questionnaire surveys of the plant employees in March. Headache and eye irritation

TABLE 9

*Personal Breathing Zone Concentrations of Hexachlorocyclopentadiene (HCCPD) and Octachlorocyclopentene (OCCP) Measured in the Grit Loading and Screen and Grit Buildings During Grit Removal, Morris Forman Wastewater Treatment Plant, Louisville, Kentucky, 1977**

Sample Date	Sample No.	Sample Description[1]	Sampling Period	Sample Volume liters	Airborne Concentration - ppb[2] HCCPD	OCCP
4 - 17	CR-022	PBZ-GLB: Maintenance Mechanic	1257 - 1322 1459 - 1533	6	1.5	2.4
4 - 18	CR-023	PBZ-SGB: Equipment Operator	1528 - 1919 2044 - 2222	12	3	1.2
"	CR-024	PBZ-SGB: Equipment Operator	1536 - 1907 2043 - 2220	16	7	0.9
4 - 19	CR-027	PBZ-GLB: Loading Operator	0805 - 0905 1008 - 1120 1243 - 1519	13	0.7	1.1
"	CR-029	PBZ-SGB: Equipment Operator	0806 - 0909 1007 - 1120 1255 - 1515	10	0.5	0.8
"	CR-035	PBZ-SGB: Equipment Operator	1645 - 1803	4	2.3	3.7
"	CR-036	PBZ-GLB: Loading Operator	1646 - 1805 2045 - 2253	10	8	2
4 - 20	CR-037	PBZ-SGB: Equipment Operator	0005 - 0119 0230 - 0440 0600 - 0740	12	6	4
"	CR-038	PBZ-GLB: Loading Operator	0006 - 0120 0235 - 0441 0600 - 0741	14	1	1.0
"	CR-041	PBZ-SGB: Equipment Operator	0747 - 0857 0950 - 1112 1303 - 1359	14	5	1.1
"	CR-042	PBZ-GLB: Loading Operator	0749 - 0851 0948 - 1113 1317 - 1405	8	7	1.9
"	CR-048	PBZ-SGB: Equipment Operator	1646 - 1715	12	0.8	1.2
"	CR-049	PBZ-GLB: Loading Operator	1858 - 2234	7	1.4	2.2
Environmental Criteria					10	None Established

*Concentration was measured underneath the protective vinyl suit in the breathing zone of the worker.

**Source: Singal, 1978

[1] PBZ-GLB denotes personal breathing zone sample in the Grit Loading Building and PBZ-SGB denotes personal breathing zone sample in the Screen and Grit Building.

[2] Parts of contaminant per billion parts of contaminated air sampled by volume.

TABLE 10

Personal Breathing Zone Concentrations of Hexachlorocyclopentadiene (HCCPD) and Octachlorocyclopentene (OCCP) Measured Inside the Protective Suits Worn by Persons Involved with the High Pressure Water Washdown of the Screen and Grit Building, Morris Forman Wastewater Treatment Plant, Louisville, Kentucky, 1977*

Sample Date	Sample No.	Sampling Period	Sample Volume liters	Airborne Concentrations - ppb[1] HCCPD	OCCP
4-22	CR-058	0945 - 1117 1225 - 1534	12	0.8	4
4-22	CR-059	0946 - 1122 1225 - 1540	15	0.6	0.9
4-22	CR-060	0947 - 1120 1226 - 1540	13	0.7	1.1
4-23	CR-078	0850 - 1145 1248 - 1600	18	0.5	0.8
4-23	CR-079	0851 - 1145 1253 - 1601	9	1.0	1.8
4-23	CR-080	1045 - 1145 1252 - 1604	8	1.0	1.4
4-25	CR-081	1245 - 1401 1438 - 1534	32	0.3	0.4
4-25	CR-082	1308 - 1405 1438 - 1537	23	0.4	0.6
4-25	CR-083	1246 - 1402	29	0.3	0.5
Environmental Criteria				10	None Established

*Source: Singal, 1978

[1]Parts of contaminant per billion parts of contaminated air sampled by volume

were the predominant symptoms; sore throat, fatigue, nausea, dizziness, chest discomfort, cough, and skin irritation were also reported. Physical examinations on cleanup crew members were unremarkable except for conjunctival irritation in workers wearing half-face respirators.

Of 97 crew members tested, 18 (19 percent) showed some elevation of a liver function test results on one or more of the five occasions testing was done (Singal, 1978). These elevations were generally small (Table 11), but once they appeared, they tended to persist over several weeks (Table 12). A small number of abnormalities appeared on renal function tests but generally these were small and non-reproducible on serial testing. Likewise, abnormalities in complete blood counts were also minor and non-reproducible.

It should be noted that the laboratory results on cleanup workers are difficult to interpret due to lack of adequate controls. Essentially all of the plant employees, including many of the cleanup workers, had been exposed in March prior to the plant shutdown. As indicated, there were no environmental samples taken at the time of the acute exposure episode. Although exposure levels of the cleanup workers were well below the current occupational standard for hex (0.01 ppm), one cannot rule out the possibility that abnormalities among the cleanup crew are reflective of earlier, unspecified exposures. Interpreting the significance of variations in liver function tests of the magnitude seen in this group of workers is difficult. First, many of the abnormalities seen are relatively nonspecific, that is such changes may be caused by a variety of conditions and thus are not necessarily

TABLE 11

Abnormalities in Lab Tests on Cleanup Workers,
Morris Forman Wastewater Treatment Plant,
Louisville, Kentucky*

Lab Test	Ranges of values	Number of Persons' Results in Range	Normal Range
1 SGOT - (serum glutamate-oxalacetic acid transaminase)	40-49	5	7-40 mU/ml
	50-59	1	
	60-69	4	
	70-79	0	
	80-89	1	
	90-99	1	
2 Serum alkaline phosphatase	100-109	3	30-100 mU/ml
	110-119	1	
	120-129	1	
3 Serum total Bilirubin	1.0-1.9	1	0.15-1.0 mg%
4 Serum LDH (lactate dehydrogenase)	230-239	1	100-225 mU/ml
5 Serum creatinine	1.3-1.9	1	0.5-1.3 mg/dl

*Source: Singal, 1978

TABLE 12

Liver Function Abnormalities in Cleanup Workers, Morris Forman Wastewater Treatment Plant, Louisville, Kentucky*

Patient No.	Date of Visit 4/8	4/12	4/20	5/5	5/19	Hours Spent in Cleanup	Did Lab Abnormality Result in Removal from Cleanup?
1				SGOT 46[1]	SGOT 35	40	Yes
				Bili 0.9[2]	Bili 1.6		
2					Alk phos[3] 117	56	Yes
3				SGOT 48	SGOT 47	115	Yes
4					SGOT 66		
					LDH[4] 239	150	Yes
5				Bili 1.4		11	No
6		SGOT 51	SGOT 63	SGOT 45	SGOT 43	100	Yes
7				Alk phos 100		5	No
8				SGOT 42			
				Alk phos 113		80	No
9	SGOT 31		SGOT 31	SGOT 60	SGOT 63	110	Yes
10	SGOT 43		SGOT 52	SGOT 39	SGOT 39	40	Yes
	Alk phos 105			Alk phos 120	Alk phos 129		
11		SGOT 44				80	No
12	Alk phos 88	Alk phos 96		Alk phos 101		60	No
13				SGOT 87	SGOT 93	80	Yes
14			Alk phos 103			15	Yes
15	LDH 232		LDH 159			32	No
16					SGOT 47	108	Yes
17			SGOT 42			40	No
18	SGOT 59		SGOT 54	SGOT 46	SGOT 48	140	Yes

*Source: Singal, 1978

1 SGOT = Serum glutamate-oxaloacetate transterase in mU/ml - Normal range = 7-40 mU/ml

2 Bili = Total serum bilirubin in mg% - Normal range = 0.15-1.0 mg%

3 Alk phos - Serum Alkaline phosphatase in mU/ml - Normal range = 30-100 mU/ml

4 LDH = Serum Lactate dehydrogenase in mU/ml - Normal range = 100-224 mU/ml

attributable to exposure. Second, there is little consensus concerning what constitutes the normal range in some of these tests. Despite these problems in analysis, Dr. Singal expressed the opinion that these data suggest that exposure to the mixture of chemicals contaminating the sewage treatment plant may be associated with some mild liver injury (Singal, 1978).

In a community survey, CDC workers administered a questionnaire to a systematically selected sample of residents in a 48-block area surrounding the contaminated sewer line (Morse, et al. 1978). One home per block was surveyed by administering a questionnaire to the head of each household. In all, 212 occupants of the 48-block area were surveyed. Questions were asked concerning basic demographic data, history of unusual odors, and any symptoms noted by household members within the past two weeks.

Results of the community survey were essentially negative. Eight of the 212 persons (3.8 percent) reported noticing an unusual odor at some time during the preceding two weeks. While some of the respondents reported symptoms compatible with hex exposure (headache, 4.7 percent; burning or watering eyes, 4.7 percent), no symptom occurred at greater than background rates. Symptoms not associated with hex were reported just as frequently as those possibly related to exposure. Furthermore, there was no association between symptom rates and distance from the sewer line. Subsequent air sampling failed to show a significant ambient concentration of hex in the sewer line area.

CRITERION FORMULATION

Existing Guidelines and Standards

The Occupational Safety and Health Administration (OSHA) has not set a standard for occupational exposure to hex. On the other hand, the American Conference of Governmental Industrial Hygienists (ACGIH) has adopted both a threshold limit value (TLV) and a Short Term Exposure Limit (STEL) for hexachlorocyclopentadiene. The current occupational TLV for hex is set at 0.01 ppm (0.11 mg/m^3), which, according to ACGIH "represents a time-weighted average concentration for a normal 8-hour workday or 40-hour workweek to which nearly all workers may be repeatedly exposed, day after day, without adverse effect" (ACGIH, 1977). The Short Term Exposure Limit (STEL) for hex is set at 0.03 ppm (0.33 mg/m^3). This level represents the maximal concentration to which workers can be exposed for a period up to 15 minutes without suffering from irritation; chronic or irreversible tissue damage; or narcosis of sufficient degree to increase accident proneness, impair self-rescue, or materially reduce work efficiency. The STEL should be considered a maximum allowable concentration or absolute ceiling not to be exceeded at any time in the 15 minutes. Up to four excursions up to the STEL are permitted per day provided that at least 60 minutes occur between excursions up to the STEL (ACGIH, 1977).

In selecting the TLV and STEL values for hex, the ACGIH emphasizes that these particular levels were selected on the basis of preventing irritant effects rather than chronic toxicity. The USSR has recommended a tenfold lower limit (0.001 ppm) for occupational exposures.

No nonoccupational exposure limits have been established or recommended except for one Soviet study which proposed a maximum concentration of 0.001 mg/l in water to prevent "organoleptic effects" (i.e., adverse effects on the taste and odor of water). There is a serious lack of data to support nonoccupational exposure limits or environmental criteria for hex. Specifically lacking are: (1) epidemiologic studies of individuals having known and quantifiable hex exposures; (2) long-term animal studies (e.g., 2-year chronic feeding studies) suitable for evaluating chronic effects, especially carcinogenicity; (3) data on current levels of human exposure from various media; and (4) suitable methods for interpreting the significance of *in vitro* assays and their applicability to actual environmental conditions. Without these essential data it is not possible to use the model proposed by U.S. EPA's Carcinogen Assessment Group (CAG) to derive recommended exposure criteria for humans. In fact, the CAG states that "there is insufficient evidence to categorize this compound as a carcinogen or noncarcinogen." Consequently, other toxic endpoints must form the basis for recommended exposure criteria until a more adequate information base on hex is developed.

Special Groups at Risk

As indicated earlier, it is presently unknown whether ingestion or inhalation of hex constitute significant sources of exposure among the general population. Although it is not likely this is the case, present data on the environmental occurrence of hex are so sketchy that this possibility cannot be ruled out.

Occupational exposures appear to constitute the only documented source of human exposure to hex. Oral contact does not appear to be a likely mode of occupational exposure. However, dermal and inhalation exposures are recognized hazards for the following groups: (1) workers engaged directly in hex manufacture; (2) those engaged in the formulation and use of other, related pesticides where hex may be present as an impurity; (3) workers dealing with flame retardants; (4) those having "quasi-occupational" exposures such as sewage treatment workers, industrial hygienists, etc.

Basis and Derivation of Criterion

Notwithstanding the obvious data deficiencies, some tentative recommendations can be made in consideration of the levels of hex which produce chronic toxicity in laboratory experiments.

As indicated earlier, there are no epidemiologic studies nor suitable chronic toxicity studies in mammals from which threshold levels for chronic effects could be derived. Very little is known regarding potential hex exposures through ingestion of contaminated food or water. In the environment hex has been detected only in specific bodies of water near points of industrial discharges. There are no data on hex levels in drinking or untreated water.

Based on the available and cited literature, there is insufficient evidence to categorize this compound as a carcinogen or non-carcinogen. There has not been a satisfactory study of the effects of chronic oral exposure to hex. A single study of chronic oral toxicity has been reported by Naishstein and Lisovskaya (1965). The test consisted of only one species (rats) and the duration of exposure was only six months. No neoplasms were reported, however

the duration of the study would not have been sufficient for a proper evaluation of carcinogenicity.

Hex has been tested for mutagenicity and reported nonmutagenic in both short-term in vitro mutagenic assays (NCI, 1977; IBT, 1977; Litton Bionetics, 1978a) and in a mouse dominant lethal study (Litton Bionetics, 1978b). No epidemiologic studies or case reports examining the relationship between exposure to hex and cancer incidences could be found in the literature. Therefore, there is virtually no information regarding the carcinogenic potential of chronic exposure to hex. In selecting hex for future chronic toxicity testing, National Cancer Institute (1977) recognized these data voids.

Although one study (Treon, et al. 1955) reported on the effects of chronic low-dose inhalation of hex, its applicability in deriving water quality guidelines is unclear. Furthermore, with the exception of very limited data on hex in water near points of discharge, there appears to be no information on hex levels in water bodies. What is needed is a method for converting the results of respiratory exposure experiments into equivalent dosages from water.

Stokinger and Woodward (1958) describe a model by which the threshold limit values (TLVs) for industrial substances in air may be used in establishing drinking water standards. The model assumes that, for any given inhaled dose, an equivalent ingested dose from ingested water can be derived using reasonable estimates of daily air and water intakes and corresponding respiratory and gastrointestinal absorption rates. In the absence of suitable chronic

ingestion studies of hex, a modified version of the Stokinger and Woodward model (44 FR 15956) can be used to estimate suitable limits for hex in water based on the established threshold limit value expressed as milligrams per cubic meter of air.

The threshold limit of 0.11 mg/m^3 (0.01 ppm) hex represents what is believed to be a maximal concentration to which a worker may be exposed for eight hours per day, five days per week over his working lifetime without hazard to health or well-being (ACGIH, 1977). Three factors are applied to the TLV to arrive at an estimate of allowable daily intake (ADI). The first factor is respiratory intake or respiratory volume during an 8-hour period (assumed to be 7.6 m^3, or one-third of the 24-hour respiratory volume of 23 m^3). The second term expresses the efficiency with which the material is absorbed from the respiratory tract. In the case of hex, as the absorption rate is unknown, 70 percent absorption is assumed. The third term is a weighting factor for converting the 5-day per week occupational exposure (inherent in the TLV) to a 7-day per week equivalent in keeping with the more continuous pattern of exposure to drinking water.

According to the model, the amount of hex that may be taken into the bloodstream and presumed to be noninjurious and which, hence, may be taken in water each day is:

$$ADI = (TLV) \times (RI) \times (RA) \times (WF)$$

where:

TLV is the industrial Threshold Limit Value, 0.11 mg/m^3 (ACGIH, 1978)

RI is the respiratory intake term (respiratory volume of 7.6 m^3 per 8 hour)

RA is the respiratory absorption coefficient, here assumed to be .70

WF is a weighting factor expressing the proportion of the week exposed to the TLV, here assumed to be 5/7 or 0.7143.

(0.11) x (7.6) x (0.70) x (0.7143) = 0.4180 mg hex per day

Therefore, ADI = 0.4180 mg

If EPA's modified version of the Stokinger and Woodward method is used (44 FR 15956), we obtain:

$$CR = ADI / [WC + (R \times F)]$$

where:

CR is the criterion for which we are solving

ADI is allowable daily intake derived from the TLV, i.e., 0.4180 mg/day

WC is the volume of water ingested per day, i.e., 2.0 liters.

R is the bioconcentration factor for hex in fish, 4.34

F is the average weight of fish consumed per day, 0.0065 kg.

Solving for CR, we obtain:

$$CR = 0.4180 / [2.0 + (4.34 \times 0.0065)]$$

CR = 0.2061 mg/l or 206 µg/l

This value is included as an example of an acceptable limit, but it is not being recommended as a criterion. According to Stokinger and Woodward (1958), "This derived value represents an approximate limiting concentration for a healthy adult population; it is only a first approximation in the development of a tentative drinking water criterion... Several adjustments in this value may be necessary. Other factors, such as taste, odor, and color may outweigh

health considerations because acceptable limits for these may be below the estimated health limit."

It should also be noted that the basis for the above recommended limit, the TLV for hex, is set on the basis of avoidance of irritation, rather than chronic effects (ACGIH, 1977). Should chronic effects data become available, both TLVs and recommendations based on them will warrant reconsideration.

A single study of chronic oral toxicity in white rats reported no adverse effects (specifically changes in peripheral blood cells, ascorbic acid content of the adrenals, conditioned reflexes of the animals, or histological structure of the organs) following daily oral administration of doses up to 0.2 µg/kg (4 µg/l) of hex in aqueous solution (Naishstein and Lisovskaya, 1965). Animals receiving the highest dosage, 2.0 µg/kg (40 µg/l), showed questionable neutropenia and lymphocytosis which the investigators thought possibly attributable to mobilization of the protective forces of the organism in response to this dose.

Naishstein and Lisovskaya (1965) determined the lowest concentrations of hex capable of altering the smell and aftertaste of water. Hex solutions were prepared by successive dilution of a saturated aqueous solution of hex (20 mg/l). This stock solution was prepared from dechlorinated tap water. The intensity of smell and aftertaste was determined from 16 and 12 observations, respectively; no indication of the number of experimental subjects was given, however. The lower confidence limit of the mean threshold response concentration was 1.4 µg/l for smell and 1.6 µg/l for aftertaste. No other experimental details were presented. Based

on these organoleptic effects, these investigators proposed a maximum permissible concentration of 1 µg/l. Stokinger and Woodward (1975) themselves noted that oftentimes "other factors, including taste, odor and color may outweigh health considerations because acceptable limits for these may be well below the estimated health limit."

Because chronic effects in a mammalian species (rats) have been documented at water concentrations of hex as low as 40 µg/l, it is obvious that an acceptable water quality criterion should be well below this level. Thus, a reasonable safety factor of 10 to 100 applied to 40 µg/l would place an appropriate criterion recommendation in the range of 4.0 - 0.4 µg/l in water. The level recommended by Naishstein and Lisovskaya (1965) based on smell and aftertaste falls well within this range.

No adverse effects on humans or mammals have been reported to be caused by hex concentrations lower than approximately 1.0 µg/l. Therefore, based on avoidance of alteration in smell and aftertaste in water, a criterion of 1.0 µg/l of hex in water is tentatively suggested. It is to be stressed that this criterion is based on inadequate chronic effects data and should be reevaluated upon completion of chronic oral toxicity studies.

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